Document made available under the Patent Cooperation Treaty (PCT)

International application number: PCT/US04/014958

International filing date: 13 May 2004 (13.05.2004)

Document type: Certified copy of priority document

Document details: Country/Office: US
Number: 60/471,294
Filing date: 15 May 2003 (15.05.2003)

Date of receipt at the International Bureau: 16 August 2004 (16.08.2004)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b)

World Intellectual Property Organization (WIPO) - Geneva, Switzerland
Organisation Mondiale de la Propriété Intellectuelle (OMPI) - Genève, Suisse

1205251

THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE

United States Patent and Trademark Office

August 09, 2004

THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A FILING DATE.

APPLICATION NUMBER: *60/471,294*
FILING DATE: *May 15, 2003*
RELATED PCT APPLICATION NUMBER: *PCT/US04/14958*

Certified by

Jon W Dudas

Acting Under Secretary of Commerce
for Intellectual Property
and Acting Director of the U.S.
Patent and Trademark Office

jc648 U.S. PTO
05/15/03

05-19 60471294 .051503

PROVISIONAL APPLICATION COVER SHEET

J1011 U.S. PTO
60/471294
05/15/03

CERTIFICATE OF EXPRESS MAILING

I hereby certify that this paper and the documents and/or fees referred to as attached therein are being deposited with the United States Postal Service on May 15, 2003 in an envelope as "Express Mail Post Office to Addressee" service under 37 CFR §1.10, Mailing Label Number EV333983971US, addressed to the Commissioner for Patents, Mail Stop Provisional Patent Application, P.O. Box 1450 Alexandria, VA 22313-1450

Lauren Kimball

Attorney Docket No.: CYTOP139P

First Named Inventor:

Mail Stop Provisional Patent Application
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir: This is a request for filing a PROVISIONAL APPLICATION under 37 CFR 1.53(c).

INVENTOR(S)/APPLICANT(S)

LAST NAME	FIRST NAME	MIDDLE INITIAL	RESIDENCE (CITY AND EITHER STATE OR FOREIGN COUNTRY)

TITLE OF INVENTION (280 characters max)

COMPOUNDS, COMPOSITIONS, AND METHODS

CORRESPONDENCE ADDRESS

Customer Number 022434

22434
PATENT AND TRADEMARK OFFICE

ENCLOSED APPLICATION PARTS (check all that apply)

☒ Specification Number of Pages 68 ☐ Other (specify) _____

☐ Drawing(s) Number of Sheets _____

☐ Assignment and Assignment Recordation Cover Sheet (recording fee of $40.00 enclosed)

☐ Applicant is entitled to Small Entity Status under 37 C.F.R. § 1.27.

☐ A check or money order is enclosed to cover the Provisional filing fees. Provisional Filing Fee Amount ($) 160

☒ The Commissioner is hereby authorized to charge any additional fees which may be required or credit any overpayment to Deposit Account No. 501339 (Order No. CYTOP138P).

Respectfully Submitted,

SIGNATURE Lauren L Stevens **DATE** May 15, 2003

TYPED NAME Lauren L. Stevens, Ph.D. **REGISTRATION NO.** 36,691

PROVISIONAL APPLICATION FILING ONLY

COMPOUNDS, COMPOSITIONS, AND METHODS

FIELD OF THE INVENTION

[0001] This invention relates to compounds which are inhibitors of the mitotic kinesin KSP and are useful in the treatment of cellular proliferative diseases, for example cancer, hyperplasias, restenosis, cardiac hypertrophy, immune disorders, fungal disorders, and inflammation.

BACKGROUND OF THE INVENTION

[0002] Among the therapeutic agents used to treat cancer are the taxanes and vinca alkaloids, which act on microtubules. Microtubules are the primary structural element of the mitotic spindle. The mitotic spindle is responsible for distribution of replicate copies of the genome to each of the two daughter cells that result from cell division. It is presumed that disruption of the mitotic spindle by these drugs results in inhibition of cancer cell division, and induction of cancer cell death. However, microtubules form other types of cellular structures, including tracks for intracellular transport in nerve processes. Because these agents do not specifically target mitotic spindles, they have side effects that limit their usefulness.

[0003] Improvements in the specificity of agents used to treat cancer is of considerable interest because of the therapeutic benefits which would be realized if the side effects associated with the administration of these agents could be reduced. Traditionally, dramatic improvements in the treatment of cancer are associated with identification of therapeutic agents acting through novel mechanisms. Examples of this include not only the taxanes, but also the camptothecin class of topoisomerase I inhibitors. From both of these perspectives, mitotic kinesins are attractive targets for new anti-cancer agents.

[0004] Mitotic kinesins are enzymes essential for assembly and function of the mitotic spindle, but are not generally part of other microtubule structures, such as in nerve processes. Mitotic kinesins play essential roles during all phases of mitosis. These

enzymes are "molecular motors" that transform energy released by hydrolysis of ATP into mechanical force which drives the directional movement of cellular cargoes along microtubules. The catalytic domain sufficient for this task is a compact structure of approximately 340 amino acids. During mitosis, kinesins organize microtubules into the bipolar structure that is the mitotic spindle. Kinesins mediate movement of chromosomes along spindle microtubules, as well as structural changes in the mitotic spindle associated with specific phases of mitosis. Experimental perturbation of mitotic kinesin function causes malformation or dysfunction of the mitotic spindle, frequently resulting in cell cycle arrest and cell death.

[0005] Among the mitotic kinesins which have been identified is KSP. KSP belongs to an evolutionarily conserved kinesin subfamily of plus end-directed microtubule motors that assemble into bipolar homotetramers consisting of antiparallel homodimers. During mitosis KSP associates with microtubules of the mitotic spindle. Microinjection of antibodies directed against KSP into human cells prevents spindle pole separation during prometaphase, giving rise to monopolar spindles and causing mitotic arrest and induction of programmed cell death. KSP and related kinesins in other, non-human, organisms, bundle antiparallel microtubules and slide them relative to one another, thus forcing the two spindle poles apart. KSP may also mediate in anaphase B spindle elongation and focussing of microtubules at the spindle pole.

[0006] Human KSP (also termed HsEg5) has been described (Blangy, et al., Cell, 83:1159-69 (1995); Whitehead, et al., Arthritis Rheum., 39:1635-42 (1996); Galgio et al., J. Cell Biol., 135:339-414 (1996); Blangy, et al., J Biol. Chem., 272:19418-24 (1997); Blangy, et al., Cell Motil Cytoskeleton, 40:174-82 (1998); Whitehead and Rattner, J. Cell Sci., 111:2551-61 (1998); Kaiser, et al., JBC 274:18925-31 (1999); GenBank accession numbers: X85137, NM004523 and U37426), and a fragment of the KSP gene (TRIP5) has been described (Lee, et al., Mol Endocrinol., 9:243-54 (1995); GenBank accession number L40372). Xenopus KSP homologs (Eg5), as well as Drosophila KLP61 F/KRP1 30 have been reported.

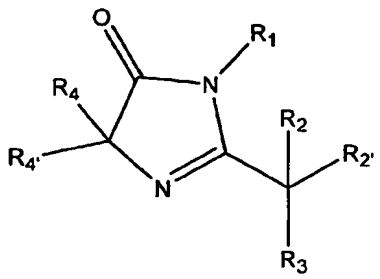
[0007] Mitotic kinesins are attractive targets for the discovery and development of novel antimitotic chemotherapeutics. Accordingly, it is an object of the present invention

to provide compounds, compositions, and methods useful in the inhibition of KSP, a mitotic kinesin.

SUMMARY OF THE INVENTION

[0008] In accordance with the objects outlined above, the present invention provides compounds, compositions and methods that can be used to treat diseases of proliferating cells. The compounds are KSP inhibitors, particularly human KSP inhibitors.

[0009] In one aspect, the invention relates to methods for treating cellular prolifcrative diseases, for treating disorders by inhibiting the activity of KSP, and for inhibiting KSP kinesin. The methods employ compounds represented by Formula I:



Formula I

wherein:

R_1 is chosen from hydrogen, optionally substituted alkyl, optionally substituted aryl, optionally substituted aralkyl, optionally substituted heteroaryl, and optionally substituted heteroaralkyl;

R_2 and R_2' are independently chosen from hydrogen, optionally substituted alkyl, optionally substituted aryl, optionally substituted aralkyl, optionally substituted heteroaryl, and optionally substituted heteroaralkyl; or R_2 and $R_{2'}$ taken together form an optionally substituted 3- to 7-membered ring;

R_3 is selected from the group consisting of optionally substituted imidazolyl, optionally substituted imidazolinyl, $-NHR_7$; $-N(R_7)(COR_6)$; $-N(R_7)(SO_2R_{6a})$; and $-N(R_7)(CH_2R_{6b})$;

R_4 and $R_{4'}$ are independently chosen from hydrogen, optionally substituted alkyl, optionally substituted aryl, optionally substituted aralkyl, optionally substituted heteroaryl, and optionally substituted heteroaralkyl; or R_4 and $R_{4'}$, together with the carbon to which they are attached form an optionally substituted 3- to 7-membered ring; or R_4 is an optionally substituted alkylidene and $R_{4'}$ is absent;

R_6 is chosen from hydrogen, optionally substituted alkyl, optionally substituted aryl, optionally substituted aralkyl, optionally substituted heteroaryl, optionally substituted heteroaralkyl, R_9O- and R_{11}-NH-;

R_{6a} is chosen from optionally substituted alkyl, optionally substituted aryl, optionally substituted aralkyl, optionally substituted heteroaryl, optionally substituted heteroaralkyl, and R_{11}-NH-;

R_{6b} is chosen from hydrogen, optionally substituted alkyl, optionally substituted aryl, optionally substituted aralkyl, optionally substituted heteroaryl, and optionally substituted heteroaralkyl;

R_7 is chosen from optionally substituted alkyl, optionally substituted aryl, optionally substituted aralkyl, optionally substituted heteroaralkyl;

R_9 is chosen from optionally substituted alkyl, optionally substituted aryl, optionally substituted aralkyl, optionally substituted heteroaryl, and optionally substituted heteroaralkyl; and

R_{11} is chosen from hydrogen, optionally substituted alkyl, optionally substituted aryl, optionally substituted aralkyl, optionally substituted heteroaryl, and optionally substituted heteroaralkyl;

including single stereoisomers and mixtures of stereoisomers; or a pharmaceutically acceptable salt of a compound of Formula I thereof; or a pharmaceutically acceptable solvate of a compound of Formula I; or a pharmaceutically acceptable solvate of a pharmaceutically acceptable salt of a compound of Formula I.

[0010] In one aspect, the invention relates to methods for treating cellular

proliferative diseases and other disorders that can be treated by inhibiting KSP kinesin activity and for inhibiting KSP by the administration of a therapeutically effective amount of a compound of Formula I or a pharmaceutically acceptable salt or solvate thereof. Diseases and disorders that respond to therapy with compounds of the invention include cancer, hyperplasia, restenosis, cardiac hypertrophy, immune disorders, fungal disorders and inflammation.

[0011] In another aspect, the invention relates to compounds useful in inhibiting KSP kinesin. The compounds have the structures shown above. The invention also relates to a pharmaceutical composition comprising a therapeutically effective amount of a compound of Formula I or a pharmaceutically acceptable salt or solvate thereof and at least one pharmaceutical excipient.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

[0012] As used in the present specification, the following words and phrases are generally intended to have the meanings as set forth below, except to the extent that the context in which they are used indicates otherwise. The following abbreviations and terms have the indicated meanings throughout:

Ac	=	acetyl
Bm	=	benzyl
Boc	=	t-butyloxy carbonyl
Bu	=	butyl
c-	=	cyclo
CBZ	=	carbobenzoxy = benzyloxycarbonyl
DCM	=	dichloromethane = methylene chloride = CH_2Cl_2
DIEA	=	N,N-diisopropylethylamine
DMF	=	N,N-dimethylformamide
DMSO	=	dimethyl sulfoxide
Et	=	ethyl
HOAc	=	acetic acid

Me	=	methyl
mesyl	=	methanesulfonyl
Ph	=	phenyl
Py	=	pyridine
rt	=	room temperature
sat'd	=	saturated
s-	=	secondary
t-	=	tertiary
TFA	=	trifluoroacetic acid
THF	=	tetrahydrofuran

[0013] **Alkyl** is intended to include linear, branched, or cyclic aliphatic hydrocarbon structures and combinations thereof, which structures may be saturated or unsaturated. **Lower-alkyl** refers to alkyl groups of from 1 to 5 carbon atoms, preferably from 1 to 4 carbon atoms. Examples of lower-alkyl groups include methyl, ethyl, propyl, isopropyl, butyl, s-and t-butyl and the like. Preferred alkyl groups are those of C_{20} or below. More preferred alkyl groups are those of C_{13} or below. **Cycloalkyl** is a subset of alkyl and includes cyclic aliphatic hydrocarbon groups of from 3 to 13 carbon atoms. Examples of cycloalkyl groups include c-propyl, c-butyl, c-pentyl, norbornyl, adamantyl and the like. **Cycloalkyl-alkyl-** is another subset of alkyl and refers to cycloalkyl attached to the parent structure through a non-cyclic alkyl. Examples of cycloalkyl-alkyl- include cyclohexylmethyl, cyclopropylmethyl, cyclohexylpropyl, and the like. In this application, alkyl includes alkanyl, alkenyl and alkynyl residues; it is intended to include vinyl, allyl, isoprenyl and the like. **Alkylene-, alkenylene-, and alkynylene-** are other subsets of alkyl, including the same residues as alkyl, but having two points of attachment within a chemical structure. Examples of alkylene include ethylene ($-CH_2CH_2-$), propylene ($-CH_2CH_2CH_2-$), dimethylpropylene ($-CH_2C(CH_3)_2CH_2-$) and cyclohexylpropylene ($-CH_2CH_2CH(C_6H_{13})-$). Likewise, examples of alkenylene include ethenylene (-CH=CH-), propenylene (-CH=CH-CH_2-), and cyclohexylpropenylene (-CH–CHCH(C_6H_{13})-). Examples of alkynylene include ethynylene (-C≡C-) and

propynylene (-CH≡CH-CH_2-). When an alkyl residue having a specific number of carbons is named, all geometric isomers having that number of carbons are intended to be encompassed; thus, for example, "butyl" is meant to include n-butyl, sec-butyl, isobutyl and t-butyl; "propyl" includes n-propyl, isopropyl, and c-propyl.

[0014] **Alkylidene** refers to the divalent group formed from an alkane by removal of two hydrogen atoms from the same carbon atom, the free valencies of which are part of a double bond, such as $(CH_3)_2C$= propan-2-ylidene.

[0015] **Alkoxy** or **alkoxyl** refers to an alkyl group, preferably including from 1 to 8 carbon atoms, of a straight, branched, or cyclic configuration, or a combination thereof, attached to the parent structure through an oxygen (i.e., the group alkyl-O-). Examples include methoxy-, ethoxy-, propoxy-, isopropoxy-, cyclopropyloxy-, cyclohexyloxy- and the like. **Lower-alkoxy** refers to alkoxy groups containing one to four carbons.

[0016] **Acyl** refers to groups of from 1 to 8 carbon atoms of a straight, branched, or cyclic configuration or a combination thereof, attached to the parent structure through a carbonyl functionality. Such groups may be saturated or unsaturated, and aliphatic or aromatic. One or more carbons in the acyl residue may be replaced by nitrogen, oxygen or sulfur as long as the point of attachment to the parent remains at the carbonyl. Examples include acetyl, benzoyl, propionyl, isobutyryl, t-butoxycarbonyl, benzyloxycarbonyl and the like. **Lower-acyl** refers to acyl groups containing one to four carbons.

[0017] **Amino** refers to the group -NH_2. The term "substituted amino" refers to the group -NHR or -NRR where each R is independently selected from the group: optionally substituted alkyl, optionally substituted alkoxy, optionally substituted amino carbonyl, optionally substituted aryl, optionally substituted heteroaryl, optionally substituted heterocyclyl, acyl, alkoxycarbonyl, sulfanyl, sulfinyl and sulfonyl, e.g., diethylamino, methylsulfonylamino, furanyl-oxy-sulfonamino.

[0018] **Aminocarbonyl-** refers to the group $-NR^cCOR^b$, $-NR^cCO_2R^a$, or $-NR^cCONR^bR^c$, where

R^a is optionally substituted C_1-C_6 alkyl, aryl, heteroaryl, aryl-C_1-C_4 alkyl-, or heteroaryl-C_1-C_4 alkyl- group;

R^b is H or optionally substituted C_1-C_6 alkyl, aryl, heteroaryl, aryl-C_1-C_4 alkyl-, or heteroaryl-C_1-C_4 alkyl- group; and

R^c is hydrogen or C_1-C_4 alkyl; and

[0019] where each optionally substituted R^b group is independently unsubstituted or substituted with one or more substituents independently selected from C_1-C_4 alkyl, aryl, heteroaryl, aryl-C_1-C_4 alkyl-, heteroaryl-C_1-C_4 alkyl-, C_1-C_4 haloalkyl, -OC_1-C_4 alkyl, -OC_1-C_4 alkylphenyl, -C_1-C_4 alkyl-OH, -OC_1-C_4 haloalkyl, halogen, -OH, $-NH_2$, -C_1-C_4 alkyl-NH_2, -N(C_1-C_4 alkyl)(C_1-C_4 alkyl), -NH(C_1-C_4 alkyl), -N(C_1-C_4 alkyl)(C_1-C_4 alkylphenyl), -NH(C_1-C_4 alkylphenyl), cyano, nitro, oxo (as a substitutent for heteroaryl), $-CO_2H$, -C(O)OC_1-C_4 alkyl, -CON(C_1-C_4 alkyl)(C_1-C_4 alkyl), -CONH(C_1-C_4 alkyl), $-CONH_2$, -NHC(O)(C_1-C_4 alkyl), -NHC(O)(phenyl), -N(C_1-C_4 alkyl)C(O)(C_1-C_4 alkyl), -N(C_1-C_4 alkyl)C(O)(phenyl), -C(O)C_1-C_4 alkyl, -C(O)C_1-C_4 phenyl, -C(O)C_1-C_4 haloalkyl, -OC(O)C_1-C_4 alkyl, $-SO_2$(C_1-C_4 alkyl), $-SO_2$(phenyl), $-SO_2$(C_1-C_4 haloalkyl), $-SO_2NH_2$, $-SO_2NH$(C_1-C_4 alkyl), $-SO_2NH$(phenyl), $-NHSO_2$(C_1-C_4 alkyl), $-NHSO_2$(phenyl), and $-NHSO_2$(C_1-C_4 haloalkyl).

[0020] **Antimitotic** refers to a drug for inhibiting or preventing mitosis, for example, by causing metaphase arrest. Some antitumour drugs block proliferation and are considered antimitotics.

[0021] **Aryl** and **heteroaryl** mean a 5- or 6-membered aromatic or heteroaromatic ring containing 0 or 1-4 heteroatoms, respectively, selected from O, N, or S; a bicyclic 9- or 10-membered aromatic or heteroaromatic ring system containing 0 or 1-4 (or more) heteroatoms, respectively, selected from O, N, or S; or a tricyclic 12- to 14-membered aromatic or heteroaromatic ring system containing 0 or 1-4 (or more) heteroatoms, respectively, selected from O, N, or S. The aromatic 6- to 14-membered carbocyclic rings include, e.g., phenyl, naphthyl, indanyl, tetralinyl, and fluorenyl and the 5- to 10-membered aromatic heterocyclic rings include, e.g., imidazolyl, pyridinyl, indolyl, thienyl, benzopyranonyl, thiazolyl, furanyl, benzimidazolyl, quinolinyl, isoquinolinyl, quinoxalinyl, pyrimidinyl, pyrazinyl, tetrazolyl and pyrazolyl.

[0022] **Aralkyl-** refers to a residue in which an aryl moiety is attached to the parent structure via an alkyl residue. Examples include benzyl, phenethyl, phenylvinyl,

phenylallyl and the like. **Heteroaralkyl-** refers to a residue in which a heteroaryl moiety is attached to the parent structure via an alkyl residue. Examples include furanylmethyl, pyridinylmethyl, pyrimidinylethyl and the like.

[0023] **Aralkoxy-** refers to the group -O-aralkyl. Similarly, **heteroaralkoxy-** refers to the group –O-heteroaralkyl; **aryloxy-** refers to the group –O-aryl; **acyloxy-** refers to the group –O-acyl; **heteroaryloxy-** refers to the group –O-heteroaryl; and **heterocyclyloxy-** refers to the group -O-heterocyclyl (i.e., aralkyl, heteroaralkyl, aryl, acyl, heterocyclyl, or heteroaryl is attached to the parent structure through an oxygen).

[0024] **Carboxyalkyl-** refers to the group –alkyl-COOH.

[0025] **Carboxamido** refers to the group $-CONR^bR^c$, where

R^b is H or optionally substituted C_1-C_6 alkyl, aryl, heteroaryl, aryl-C_1-C_4 alkyl-, or heteroaryl-C_1-C_4 alkyl- group; and

R^c is hydrogen or C_1-C_4 alkyl; and

where each optionally substituted R^b group is independently unsubstituted or substituted with one or more substituents independently selected from C_1-C_4 alkyl, aryl, heteroaryl, aryl-C_1-C_4 alkyl-, heteroaryl-C_1-C_4 alkyl-, C_1-C_4 haloalkyl, -OC_1-C_4 alkyl, -OC_1-C_4 alkylphenyl, -C_1-C_4 alkyl-OH, -OC_1-C_4 haloalkyl, halogen, -OH, -NH_2, -C_1-C_4 alkyl-NH_2, -N(C_1-C_4 alkyl)(C_1-C_4 alkyl), -NH(C_1-C_4 alkyl), -N(C_1-C_4 alkyl)(C_1-C_4 alkylphenyl), -NH(C_1-C_4 alkylphenyl), cyano, nitro, oxo (as a substitutent for heteroaryl), -CO_2H, -C(O)OC_1-C_4 alkyl, -CON(C_1-C_4 alkyl)(C_1-C_4 alkyl), -CONH(C_1-C_4 alkyl), -$CONH_2$, -NHC(O)(C_1-C_4 alkyl), -NHC(O)(phenyl), -N(C_1-C_4 alkyl)C(O)(C_1-C_4 alkyl), -N(C_1-C_4 alkyl)C(O)(phenyl), -C(O)C_1-C_4 alkyl, -C(O)C_1-C_4 phenyl, -C(O)C_1-C_4 haloalkyl, -OC(O)C_1-C_4 alkyl, -SO_2(C_1-C_4 alkyl), -SO_2(phenyl), -SO_2(C_1-C_4 haloalkyl), -SO_2NH_2, -SO_2NH(C_1-C_4 alkyl), -SO_2NH(phenyl), -$NHSO_2$(C_1-C_4 alkyl), -$NHSO_2$(phenyl), and -$NHSO_2$(C_1-C_4 haloalkyl).

[0026] **Halogen** or **halo** refers to fluorine, chlorine, bromine or iodine. Fluorine, chlorine and bromine are preferred. Dihaloaryl, dihaloalkyl, trihaloaryl etc. refer to aryl and alkyl substituted with the designated plurality of halogens (here, 2, 2 and 3, respectively), but not necessarily a plurality of the same halogen; thus 4-chloro-3-fluorophenyl is within the scope of dihaloaryl.

[0027] **Heterocyclyl** means a cycloalkyl or aryl residue in which one to four of the carbons is replaced by a heteroatom such as oxygen, nitrogen or sulfur. Examples of heterocycles that fall within the scope of the invention include azetidinyl, imidazolinyl, pyrrolidinyl, pyrazolyl, pyrrolyl, indolyl, quinolinyl, isoquinolinyl, tetrahydroisoquinolinyl, benzofuranyl, benzodioxanyl, benzodioxyl (commonly referred to as methylenedioxyphenyl, when occurring as a substituent), tetrazolyl, morpholinyl, thiazolyl, pyridinyl, pyridazinyl, piperidinyl, pyrimidinyl, thienyl, furanyl, oxazolyl, oxazolinyl, isoxazolyl, dioxanyl, tetrahydrofuranyl and the like. "N-heterocyclyl" refers to a nitrogen-containing heterocycle. The term heterocyclyl encompasses heteroaryl, which is a subset of heterocyclyl. Examples of N-heterocyclyl residues include azetidinyl, 4-morpholinyl, 4-thiomorpholinyl, 1-piperidinyl, 1-pyrrolidinyl, 3-thiazolidinyl, piperazinyl and 4-(3,4-dihydrobenzoxazinyl). Examples of substituted heterocyclyl include 4-methyl-1-piperazinyl and 4-benzyl-1-piperidinyl.

[0028] A **leaving group** or **atom** is any group or atom that will, under the reaction conditions, cleave from the starting material, thus promoting reaction at a specified site. Suitable examples of such groups unless otherwise specified are halogen atoms, mesyloxy, p-nitrobenzensulphonyloxy and tosyloxy groups.

[0029] **Optional** or **optionally** means that the subsequently described event or circumstance may or may not occur, and that the description includes instances where said event or circumstances occurs and instances in which it does not. For example, "optionally substituted alkyl" includes "alkyl" and "substituted alkyl" as defined herein. It will be understood by those skilled in the art with respect to any group containing one or more substituents that such groups are not intended to introduce any substitution or substitution patterns that are sterically impractical and/or synthetically non-feasible and/or inherently unstable.

[0030] **Substituted alkoxy** refers to alkoxy wherein the alkyl constituent is substituted (i.e., -O-(substituted alkyl)). One preferred substituted alkoxy group is "polyalkoxy" or -O-(optionally substituted alkylene)-(optionally substituted alkoxy), and includes groups such as $-OCH_2CH_2OCH_3$, and residues of glycol ethers such as polyethyleneglycol, and $-O(CH_2CH_2O)_xCH_3$, where x is an integer of about 2-20,

preferably about 2-10, and more preferably about 2-5. Another preferred substituted alkoxy group is hydroxyalkoxy or $-OCH_2(CH_2)_yOH$, where y is an integer of about 1-10, preferably about 1-4.

[0031] **Substituted** alkyl, aryl, and heteroaryl, which includes the substituted alkyl, aryl and heteroaryl moieties of any group containing an optionally substituted alkyl, aryl and heteroaryl moiety (e.g., alkoxy, aralkyl and heteroaralkyl), refer respectively to alkyl, aryl, and heteroaryl wherein one or more (up to about 5, preferably up to about 3) hydrogen atoms are replaced by a substituent independently selected from the group:

$-R^a$, $-OR^b$, $-O(C_1-C_2$ alkyl$)O-$ (as an aryl substituent), $-SR^b$, $-NR^bR^c$, halogen, cyano, nitro, $-COR^b$, $-CO_2R^b$, $-CONR^bR^c$, $-OCOR^b$, $-OCO_2R^a$, $-OCONR^bR^c$, $-NR^cCOR^b$, $-NR^cCO_2R^a$, $-NR^cCONR^bR^c$, $-CO_2R^b$, $-CONR^bR^c$, $-NR^cCOR^b$, $-SOR^a$, $-SO_2R^a$, $-SO_2NR^bR^c$, and $-NR^cSO_2R^a$,

where R^a is an optionally substituted C_1-C_6 alkyl, aryl, heteroaryl, aryl-C_1-C_4 alkyl-, or heteroaryl-C_1-C_4 alkyl- group, R^b is H or optionally substituted C_1-C_6 alkyl, aryl, heteroaryl, aryl-C_1-C_4 alkyl-, or heteroaryl-C_1-C_4 alkyl- group;

R^c is hydrogen or C_1-C_4 alkyl;

where each optionally substituted R^a group and R^b group is independently unsubstituted or substituted with one or more substituents independently selected from C_1-C_4 alkyl, aryl, heteroaryl, aryl-C_1-C_4 alkyl-, heteroaryl-C_1-C_4 alkyl-, C_1-C_4 haloalkyl, $-OC_1-C_4$ alkyl, $-OC_1-C_4$ alkylphenyl, $-C_1-C_4$ alkyl-OH, $-OC_1-C_4$ haloalkyl, -OH, $-NH_2$, $-C_1-C_4$ alkyl-NH_2, $-N(C_1-C_4$ alkyl$)(C_1-C_4$ alkyl$)$, $-NH(C_1-C_4$ alkyl$)$, $-N(C_1-C_4$ alkyl$)(C_1-C_4$ alkylphenyl$)$, $-NH(C_1-C_4$ alkylphenyl$)$, amidino, imidate, heterocyclyl-, guanidine, cyano, nitro, oxo (as a substitutent for heteroaryl), $-CO_2H$, $-C(O)OC_1-C_4$ alkyl, $-CON(C_1-C_4$ alkyl$)(C_1-C_4$ alkyl$)$, $-CONH(C_1-C_4$ alkyl$)$, $-CONH_2$, $-NHC(O)(C_1-C_4$ alkyl$)$, -NHC(O)(phenyl), $-N(C_1-C_4$ alkyl$)C(O)(C_1-C_4$ alkyl$)$, $-N(C_1-C_4$ alkyl$)C(O)$(phenyl), $-C(O)C_1-C_4$ alkyl, $-C(O)C_1-C_4$ phenyl, $-C(O)C_1-C_4$ haloalkyl, $-OC(O)C_1-C_4$ alkyl, $-S(C_1-C_4$ alkyl$)$, $-S(C_1-C_4$ aryl$)$, $-SO_2(C_1-C_4$ alkyl$)$, $-SO_2$(phenyl), $-SO_2(C_1-C_4$ haloalkyl$)$, $-SO_2NH_2$, $-SO_2NH(C_1-C_4$ alkyl$)$, $-SO_2NH$(phenyl), $-NHSO_2(C_1-C_4$ alkyl$)$, $-NHSO_2$(phenyl), and $-NHSO_2(C_1-C_4$ haloalkyl$)$.

[0032] **Sulfanyl** refers to the groups: -S-(optionally substituted alkyl),

-S-(optionally substituted aryl), -S-(optionally substituted heteroaryl), and -S-(optionally substituted heterocyclyl).

[0033] **Sulfinyl** refers to the groups: -S(O)-H, -S(O)-(optionally substituted alkyl), -S(O)-optionally substituted aryl), -S(O)-(optionally substituted heteroaryl), -S(O)-(optionally substituted heterocyclyl); and -S(O)-(optionally substituted amino).

[0034] **Sulfonyl** refers to the groups: -$S(O_2)$-H, -$S(O_2)$-(optionally substituted alkyl), -$S(O_2)$-optionally substituted aryl), -$S(O_2)$-(optionally substituted heteroaryl), -$S(O_2)$-(optionally substituted heterocyclyl) ,-$S(O_2)$-(optionally substituted alkoxy), -$S(O_2)$-optionally substitutcd aryloxy), -$S(O_2)$-(optionally substituted heteroaryloxy), -$S(O_2)$-(optionally substituted heterocyclyloxy); and -$S(O_2)$-(optionally substituted amino).

[0035] **Pharmaceutically acceptable salts** refers to those salts that retain the biological effectiveness of the free compound and that are not biologically or otherwise undesirable, formed with a suitable acid or base, and includes pharmaceutically acceptable acid addition salts and base addition salts. **Pharmaceutically acceptable acid addition salts** include those derived from inorganic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid and the like, and those derived from organic acids such as acetic acid, propionic acid, glycolic acid, pyruvic acid, oxalic acid, maleic acid, malonic acid, succinic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, p-toluenesulfonic acid, salicylic acid and the like.

[0036] **Pharmaceutically acceptable base addition salts** include those derived from inorganic bascs such as sodium, potassium, lithium, ammonium, calcium, magncsium, iron, zinc, copper, manganese, aluminum salts and the like. Particularly preferred are the ammonium, potassium, sodium, calcium, and magnesium salts. Base additioin salts also include those derived from pharmaceutically acceptable organic non-toxic bases, includingsalts of primary, secondary, and tertiary amines, substituted amines including naturally occurring substituted amines, cyclic amines and basic ion exchange resins, such as isopropylamine, trimethylamine, diethylamine, triethylamine, tripropylamine, and ethanolamine.

[0037] **Protecting group** has the meaning conventionally associated with it in organic synthesis, i.e. a group that selectively blocks one or more reactive sites in a multifunctional compound such that a chemical reaction can be carried out selectively on another unprotected reactive site and such that the group can readily be removed after the selective reaction is complete. A variety of protecting groups are disclosed, for example, in T.H. Greene and P. G. M. Wuts, Protective Groups in Organic Synthesis, Third Edition, John Wiley & Sons, New York (1999), which is incorporated herein by reference in its entirety. For example, a hydroxy protected form is where at least one of the hydroxyl groups present in a compound is protected with a hydroxy protecting group. Likewise, amines and other reactive groups may similarly be protected.

[0038] **Solvate** refers to the compound formed by the interaction of a solvent and a compound of Formula I or salt thereof. Suitable solvates of the compounds of the Formula I are pharmaceutically acceptable solvates, such as hydrates, including monohydrates and hemi-hydrates.

[0039] Many of the compounds described herein contain one or more asymmetric centers (e.g. the carbon to which R_2 and $R_{2'}$ are attached) and may thus give rise to enantiomers, diastereomers, and other stereoisomeric forms that may be defined, in terms of absolute stereochemistry, as (R)- or (S)-. The present invention is meant to include all such possible isomers, including racemic mixtures, optically pure forms and intermediate mixtures. Optically active (R)- and (S)- isomers may be prepared using chiral synthons or chiral reagents, or resolved using conventional techniques. When the compounds described herein contain olefinic double bonds or other centers of geometric asymmetry, and unless specified otherwise, it is intended that the compounds include both E and Z geometric isomers. Likewise, all tautomeric forms and rotational isomers are also intended to be included.

[0040] When desired, the R- and S-isomers may be resolved by methods known to those skilled in the art, for example by formation of diastereoisomeric salts or complexes which may be separated, for example, by crystallisation; via formation of diastereoisomeric derivatives which may be separated, for example, by crystallisation, gas-liquid or liquid chromatography; selective reaction of one enantiomer with an

enantiomer-specific reagent, for example enzymatic oxidation or reduction, followed by separation of the modified and unmodified enantiomers; or gas-liquid or liquid chromatography in a chiral environment, for example on a chiral support, such as silica with a bound chiral ligand or in the presence of a chiral solvent. It will be appreciated that where the desired enantiomer is converted into another chemical entity by one of the separation procedures described above, a further step may be required to liberate the desired enantiomeric form. Alternatively, specific enantiomer may be synthesized by asymmetric synthesis using optically active reagents, substrates, catalysts or solvents, or by converting one enantiomer to the other by asymmetric transformation.

Compounds of the Present Invention

[0041] The present invention is directed to a class of novel compounds that are inhibitors of mitotic kinesins. By inhibiting mitotic kinesins, but not other kinesins (e.g., transport kinesins), specific inhibition of cellular proliferation is accomplished. While not intending to be bound by any theory, the present invention capitalizes on the finding that perturbation of mitotic kinesin function causes malformation or dysfunction of mitotic spindles, frequently resulting in cell cycle arrest and cell death.

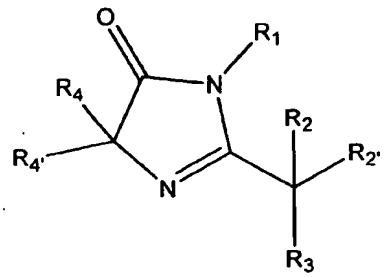
[0042] According to one embodiment of the invention, the compounds described herein inhibit the mitotic kinesin, KSP. In another embodiment, the compounds inhibit the mitotic kinesin, KSP, as well as modulating one or more of the human mitotic kinesins selected from the group consisting of HSET (see, U.S. Patent No. 6,361,993, which is incorporated herein by reference); MCAK (see, U.S. Patent No. 6,331,424, which is incorporated herein by reference); CENP-E (see, PCT Publication No. WO99/13061 , which is incorporated herein by reference); Kif4 (see, U.S. Patent No. 6,440,684, which is incorporated herein by reference); MKLP1 (see, U.S. Patent No. 6,448,025, which is incorporated herein by reference); Kif15 (see, U.S. Patent No. 6,355,466, which is incorporated herein by reference); Kid (see, U.S. Patent No. 6,387,644, which is incorporated herein by reference); Mpp1, CMKrp, KinI-3 (see, U.S. Patent No. 6,461,855, which is incorporated herein by reference); Kip3a (see, PCT Publication No. WO 01/96593, which is incorporated herein by reference); Kip3d (see,

U.S. Patent No. 6,492,151, which is incorporated herein by reference); and RabK6.

[0043] The methods of inhibiting a human kinesin comprise contacting an inhibitor of the invention with a human kinesin, particularly human KSP kinesins, including fragments and variants of KSP. The inhibition can be of the ATP hydrolysis activity of the KSP kinesin and/or the mitotic spindle formation activity, such that the mitotic spindles are disrupted. Meiotic spindles may also be disrupted.

[0044] The present invention provides inhibitors of mitotic kinesins, in particular KSP and especially human KSP, for the treatment of disorders associated with cell proliferation. Traditionally, dramatic improvements in the treatment of cancer, one type of cellular proliferative disorder, have been associated with identification of therapeutic agents acting through novel mechanisms. Examples of this include not only the taxane class of agents that appear to act on microtubule formation, but also the camptothecin class of topoisomerase I inhibitors. The compounds, compositions and methods described herein can differ in their selectivity and are used to treat diseases of cellular proliferation, including, but not limited to cancer, hyperplasias, restenosis, cardiac hypertrophy, immune disorders, fungal disorders and inflammation.

[0045] Accordingly, the present invention relates to methods employing compounds represented by Formula I:



Formula I

wherein:

R_1 is chosen from hydrogen, optionally substituted alkyl, optionally substituted

aryl, optionally substituted aralkyl, optionally substituted heteroaryl, and optionally substituted heteroaralkyl;

R_2 and R_2' are independently chosen from hydrogen, optionally substituted alkyl, optionally substituted aryl, optionally substituted aralkyl, optionally substituted heteroaryl, and optionally substituted heteroaralkyl; or R_2 and $R_{2'}$ taken together form an optionally substituted 3- to 7-membered ring;

R_3 is selected from the group consisting of optionally substituted imidazolyl, optionally substituted imidazolinyl, $-NHR_7$; $-N(R_7)(COR_6)$; $-N(R_7)(SO_2R_{6a})$; and $-N(R_7)(CH_2R_{6b})$;

R_4 and $R_{4'}$ are independently chosen from hydrogen, optionally substituted alkyl, optionally substituted aryl, optionally substituted aralkyl, optionally substituted heteroaryl, and optionally substituted heteroaralkyl; or R_4 and $R_{4'}$, together with the carbon to which they are attached form an optionally substituted 3- to 7-membered ring; or R_4 is an optionally substituted alkylidene and $R_{4'}$ is absent;

R_6 is chosen from hydrogen, optionally substituted alkyl, optionally substituted aryl, optionally substituted aralkyl, optionally substituted heteroaryl, optionally substituted heteroaralkyl, R_9O- and R_{11}-NH-;

R_{6a} is chosen from optionally substituted alkyl, optionally substituted aryl, optionally substituted aralkyl, optionally substituted heteroaryl, optionally substituted heteroaralkyl, and R_{11}-NH-;

R_{6b} is chosen from hydrogen, optionally substituted alkyl, optionally substituted aryl, optionally substituted aralkyl, optionally substituted heteroaryl, and optionally substituted heteroaralkyl;

R_7 is chosen from optionally substituted alkyl, optionally substituted aryl, optionally substituted aralkyl, optionally substituted heteroaralkyl;

R_9 is chosen from optionally substituted alkyl, optionally substituted aryl, optionally substituted aralkyl, optionally substituted heteroaryl, and optionally substituted heteroaralkyl; and

R_{11} is chosen from hydrogen, optionally substituted alkyl, optionally substituted aryl, optionally substituted aralkyl, optionally substituted heteroaryl, and optionally

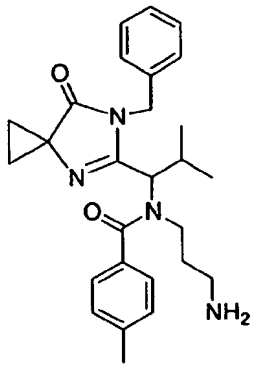
substituted heteroaralkyl;

including single stereoisomers and mixtures of stereoisomers; or a pharmaceutically acceptable salt of a compound of Formula I thereof; or a pharmaceutically acceptable solvate of a compound of Formula I; or a pharmaceutically acceptable solvate of a pharmaceutically acceptable salt of a compound of Formula I.

[0046] In a particular embodiment, the stereogenic center to which R_2 and $R_{2'}$ are attached is of the R configuration.

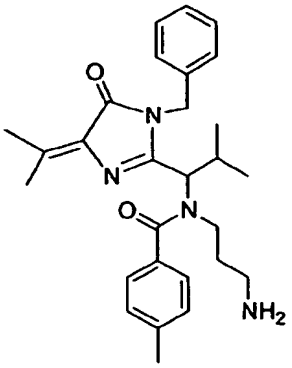
Nomenclature

[0047] The compounds of Formula I can be named and numbered in the manner (e.g., using AutoNom version 2.1 in ChemDraw or ISIS-DRAW) described below. For example, the compound



i.e., the compound according to Formula I where R_1 is benzyl, R_2 is propyl (or i -propyl), $R_{2'}$ is hydrogen; R_3 is $-N(R_7)(COR_6)$; R_4 and $R_{4'}$, together with the carbon to which they are attached, form a cyclopropyl ring; R_7 is 3-aminopropyl; and R_6 is p-tolyl can be named N-(3-amino-propyl)-N-[1-(6-benzyl-7-oxo-4,6-diaza-spiro[2.4]hept-4-en-5-yl)-2-methyl-propyl]-4-methyl-benzamide.

[0048] Likewise, the compound having the structure



i.e., the compound according to Formula I where R_1 is benzyl, R_2 is propyl (or i -propyl), $R_{2'}$ is hydrogen; R_3 is $-N(R_7)(COR_6)$; R_4 is an alkylidene group with R_5 and $R_{5'}$ being methyl; $R_{4'}$ is absent; R_7 is 3-aminopropyl; and R_6 is p-tolyl can be named N-(3-amino-propyl)-N-[1-(1-benzyl-4-isopropylidene-5-oxo-4,5-dihydro-1H-imidazol-2-yl)-2-methyl-propyl]-4-methyl-benzamide.

Synthetic Reaction Parameters

[0049] The compounds of Formula I can be prepared by following the procedures described with reference to the Reaction Schemes below or utilizing techniques well known in the art.

[0050] Unless specified otherwise, the terms "solvent", "inert organic solvent" or "inert solvent" mean a solvent inert under the conditions of the reaction being described in conjunction therewith [including, for example, benzene, toluene, acetonitrile, tetrahydrofuran ("THF"), dimethylformamide ("DMF"), chloroform, methylene chloride (or dichloromethane), diethyl ether, methanol, pyridine and the like]. Unless specified to the contrary, the solvents used in the reactions of the present invention are inert organic solvents.

[0051] The term "q.s." means adding a quantity sufficient to achieve a stated function, e.g., to bring a solution to the desired volume (i.e., 100%).

[0052] In general, esters of carboxylic acids may be prepared by conventional esterification procedures, for example alkyl esters may be prepared by treating the

required carboxylic acid with the appropriate alkanol, generally under acidic conditions. Likewise, amides may be prepared using conventional amidation procedures, for example amides may be prepared by treating the relevant activated carboxylic acid with the appropriate amine. Alternatively, a lower-alkyl ester such as a methyl ester of the acid may be treated with an amine to provide the required amide, optionally in presence of trimethylalluminium following the procedure described in Tetrahedron Lett. 48, 4171-4173, (1977). Carboxyl groups may be protected as alkyl esters, for example methyl esters, which esters may be prepared and removed using conventional procedures, one convenient method for converting carbomcthoxy to carboxyl is to use aqueous lithium hydroxidc.

[0053] The salts and solvates of the compounds mentioned herein may as required be produced by methods conventional in the art. For example, if an inventive compound is an acid, a desired base addition salt can be prepared by treatment of the free acid with an inorganic or organic base, such as an amine (primary, secondary, or tertiary); an alkali metal or alkaline earth metal hydroxide; or the like. Illustrative examples of suitable salts include organic salts derived from amino acids such as glycine and arginine; ammonia; primary, secondary, and tertiary amines; such as ethylenediamine, and cyclic amines, such as cyclohexylamine, piperidine, morpholine, and piperazine; as well as inorganic salts derived from sodium, calcium, potassium, magnesium, manganese, iron, copper, zinc, aluminum, and lithium.

[0054] If a compound is a base, a desired acid addition salt may be prepared by any suitable method known in the art, including treatment of the free base with an inorganic acid, such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, and the like, or with an organic acid, such as acetic acid, maleic acid, succinic acid, mandelic acid, fumaric acid, malonic acid, pyruvic acid, oxalic acid, glycolic acid, salicylic acid, pyranosidyl acid, such as glucuronic acid or galacturonic acid, alpha-hydroxy acid, such as citric acid or tartaric acid, amino acid, such as aspartic acid or glutamic acid, aromatic acid, such as benzoic acid or cinnamic acid, sulfonic acid, such as p-toluenesulfonic acid, methanesulfonic acid, ethanesulfonic acid, or the like.

[0055] Isolation and purification of the compounds and intermediates described

herein can be affected, if desired, by any suitable separation or purification procedure such as, for example, filtration, extraction, crystallization, column chromatography, thin-layer chromatography or thick-layer chromatography, or a combination of these procedures. Specific illustrations of suitable separation and isolation procedures can be had by reference to the examples hereinbelow. However, other equivalent separation or isolation procedures can, of course, also be used.

Synthesis of the Compounds of Formula I

[0056] The compounds of Formula I can be prepared by following the procedures described with reference to the Reaction Schemes below.

Brief Description Of Reaction Schemes

[0057] Reaction Scheme 1 illustrates a synthesis of compounds of Formula I.

[0058] Reaction Scheme 2 illustrates a synthesis of compounds of Formula I where R_4 is an optionally substituted alkylidene group and $R_{4'}$ is absent.

[0059] Reaction Scheme 3 illustrates a synthesis of compounds of Formula I wherein R_3 is $-N(R_7)(SO_2R_{6a})$.

[0060] Reaction Scheme 4 illustrates a synthesis of compounds of Formula I wherein R_3 is $-N(R_7)(CH_2R_{6b})$.

[0061] Reaction Scheme 5 illustrates a synthesis of compounds of Formula I wherein R_3 is an optionally substituted imidazolyl.

[0062] Reaction Scheme 6 illustrates another synthesis of compounds of Formula I wherein R_3 is an optionally substituted imidazolyl.

[0063] Reaction Scheme 7 illustrates a synthesis of compounds of Formula I wherein R_3 is optionally substituted imidazolinyl.

[0064] Reaction Scheme 8 illustrates a second synthesis of compounds of Formula I wherein R_3 is optionally substituted imidazolinyl.

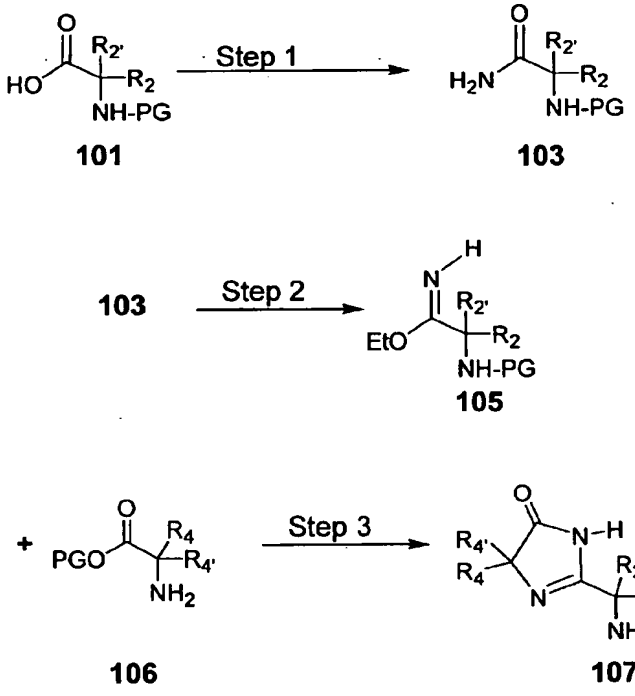
[0065] Reaction Scheme 9 illustrates a synthesis of compounds of Formula I wherein R_3 is $-N(R_7)(COR_6)$ wherein R_6 is $-OR_9$.

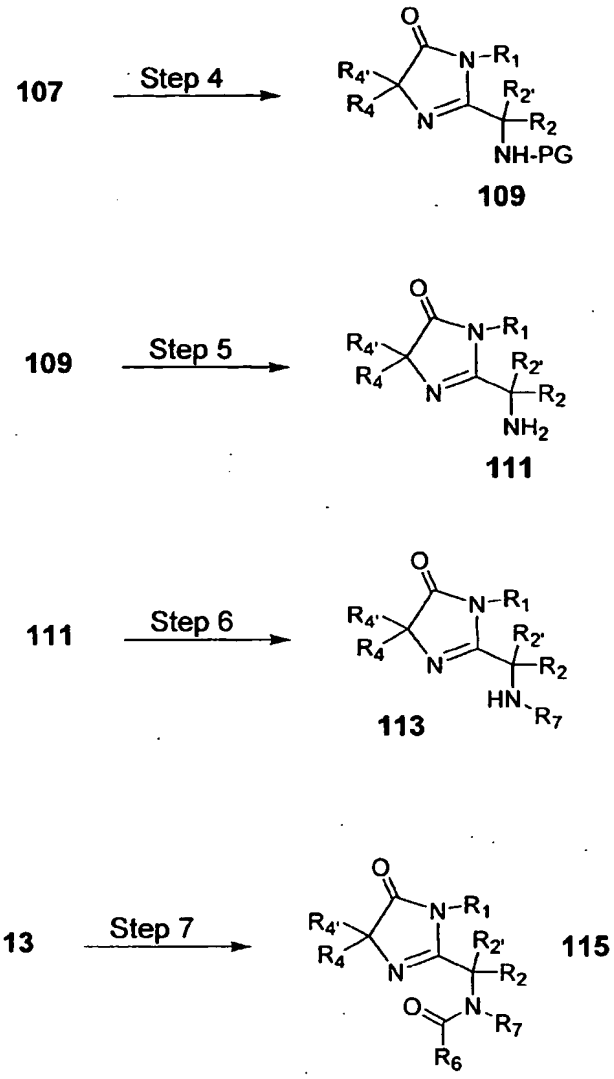
[0066] Reaction Scheme 10 illustrates a synthesis of compounds of Formula I wherein R_3 is $-N(R_7)(COR_6)$ wherein R_6 is $-NHR_{11}$.

Starting Materials

[0067] The optionally substituted compounds of Formula 101 as well as the other reactants are commercially available, e.g., from Aldrich Chemical Company, Milwaukee, WI or may be readily prepared by those skilled in the art using commonly employed synthetic methodology.

Reaction Scheme 1





Preparation of Compounds of Formula 103

[0068] Referring to Reaction Scheme 1, Step 1, a compound of Formula 101 (preferably, wherein the amino protecting PG, is CBZ); an excess (preferably about 1.1 equivalents) of ethyl chloroformate; and a base such as triethylamine in a nonpolar, aprotic solvent such as THF is cooled to about 0 °C. The reaction mixture is stirred under nitrogen. After about 1 h, the flask is equipped with a dry-ice reflux condenser and purged continuously with ammonia gas for about 30 minutes. The reaction mixture is

stirred for about an additional 1 h. The product, a compound of Formula 103, is isolated and used without further purification.

Preparation of Compounds of Formula 105

[0069] Referring to Reaction Scheme 1, Step 2, to a suspension of a compound of Formula 103 in a nonpolar, aprotic solvent such as dichloromethane is added an excess (preferably about 1.7 equivalents) of triethyloxonium hexafluorophosphate. The resulting mixture is stirred for about 3 days. The product, a compound of Formula 105, is isolated and used in the next step without further purification.

Preparation of Compounds of Formula 107

[0070] Referring to Reaction Scheme 1, Step 3, to a solution of a compound of Formula 105 in a nonpolar, aprotic solvent such as toluene are added a compound of Formula 106 (preferably as the corresponding methyl or ethyl ester) and a base such as *N*, *N*-diisopropylethylamine. The resulting mixture is refluxed for about 8 h. Acetic acid is added. Refluxing is continued for about 2 h. The product, a compound of Formula 107, is isolated and purified.

Preparation of Compounds of Formula 109

[0071] Referring to Reaction Scheme 1, Step 4, to a solution of a compound of Formula 107 in a polar, aprotic solvent such as DMF are added an excess of a compound of Formula R_1-X (wherein X is a leaving group and more preferably, is a halide) and a base such as K_2CO_3. The resulting mixture is stirred for about 3 h. The product, a compound of Formula 109, is isolated and used in the next step without further purification.

Preparation of Compounds of Formula 111

[0072] Referring to Reaction Scheme 1, Step 5, the amino protecting group is then removed. When PG is CBZ, this can be accomplished as follows. A solution of a compound of Formula 109 in a polar, protic solvent such as methanol is stirred under a

stream of H_2 (at about 30 psi) in the presence of 10% Pd/C for about 1 h. The catalyst is removed by filtration through a filter. The product, a compound of Formula 111, is isolated and used in the next step without further purification.

Preparation of Compounds of Formula 113

[0073] Referring to Reaction Scheme 1, Step 6, to a solution of a compound of Formula 111 in a nonpolar, aprotic solvent such as dichloromethane at about 0°C is added sodium triacetoxyborohydride and an excess (preferably about 1.4 equivalents) of an aldehyde comprising R_7, (i.e., a compound having the formula R_7,CHO where R_7,CH_2- is equivalent to R_7 and R_7 is as described above or is a protected precursor to such a substituent, e.g., (3-oxo-propyl)-carbamic acid *tert*-butyl ester). The resulting mixture is stirred under nitrogen for about 2 h. Additional aldehyde and sodium triacetoxyborohydride are added. Stirring is continued for an additional 1 h. The product, a compound of Formula 113, is isolated and used in the next step without purification.

Preparation of Compounds of Formula 115

[0074] Referring to Reaction Scheme 1, Step 7, to a solution of a compound of Formula 113 in a nonpolar, aprotic solvent such as dichloromethane at about 0°C are added a base such as DIEA and an excess (preferably about 1.1 equivalents) of an acid chloride of Formula R_6-(CO)-Cl. The resulting solution is stirred under nitrogen at room temperature for about 14 hours. The product, a compound of Formula 115, is isolated and purified.

[0075] In an embodiment wherein R_7 further comprises a protected amine, the protecting group may be removed. For example, when the amino protecting group is Boc, this may be accomplished by treating a solution of a compound of Formula 115 in a nonpolar, aprotic solvent such as CH_2Cl_2 with trifluoroacetic acid. The product, the corresponding free amine, is isolated and purified.

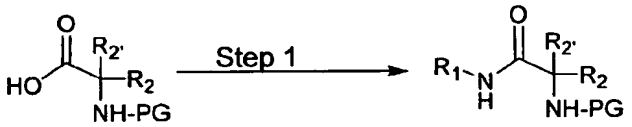
Preparation of Optically Active Compounds

[0076] In certain compounds of the invention, a particular stereo configuration

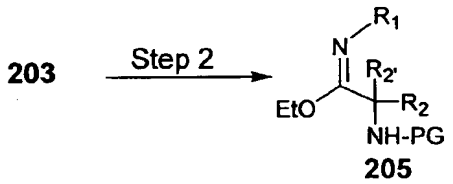
(such as the (R) isomer) may be preferred at the stereogenic center to which R_2 or to which $R_4/R_{4'}$ is attached. The optically active compound can be prepared from the racemic mixture by methods known in the art. For example, an amine of Formula 111 is dissolved in an inert organic solvent (such as IPA) and warmed to 60°C. In a separate vessel, a resolving agent (such as dibenzoyl-D-tartaric acid) is dissolved, preferably in the same warm solvent, and then quickly added (with agitation) to the warm amine solution. The reaction mixture is left to crystallize by cooling to room temperature over about 16 hours under continuing agitation. The desired isomer, e.g., the (R) isomer, is isolated and purified.

[0077] For the sake of brevity in the remaining description of the synthesis of compounds of Formula I, it should be understood that either single isomer or a mixture of isomers may be employed to give the corresponding product.

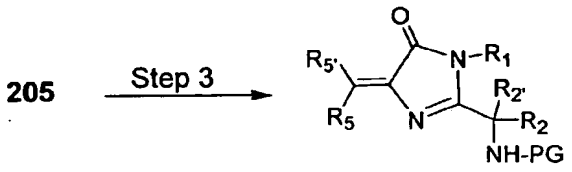
Reaction Scheme 2



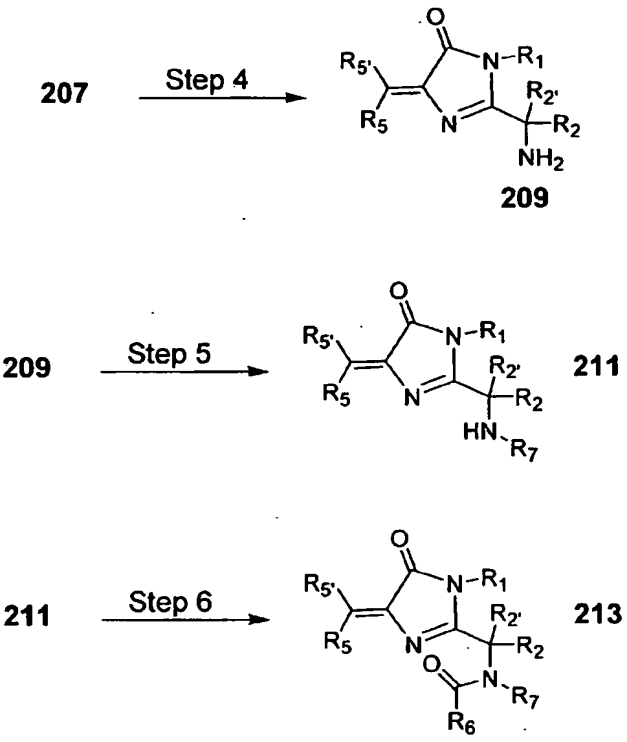
101 **203**



205



207



Preparation of Compounds of Formula 203

[0078] Referring to Reaction Scheme 2, Step 1, to a solution of a compound of Formula 101, preferably wherein the amine protecting group, PG, is CBZ, in a nonpolar, protic solvent such as THF are added an excess (preferably, about 1.2 equivalents) of ethyl chloroformate and a base such as triethylamine at about 0 °C. The reaction mixture is stirred under nitrogen. After about 1 h, an excess (preferably, about 1.2 equivalents) of a compound of Formula R_1-NH_2 is added over about 5 minutes. The product, a compound of Formula 203, is isolated and used without further purification.

Preparation of Compounds of Formula 205

[0079] Referring to Reaction Scheme 2, Step 2, to a suspension of a compound of Formula 203 in a nonpolar, aprotic solvent such as dichloromethane is added an excess (preferably about 1.7 equivalents) of triethyloxonium hexafluorophosphate. The resulting mixture is stirred for about 14 h. The product, a compound of Formula 205, is isolated

and used in the next step without further purification.

Preparation of Compounds of Formula 207

[0080] Referring to Reaction Scheme 2, Step 3, to a solution of a compound of Formula 205 in a nonpolar solvent such as toluene and a compound having the formula $R_5(CO)R_{5'}$ (wherein R_5 and $R_{5'}$ are as described below) are added an excess (preferably about two equivalents) of glycine methyl ester hydrochloride and a base such as *N*, *N*-diisopropylethylamine. The resulting mixture is refluxed for about 20 h. The product, a compound of Formula 207, is isolated and purified.

Preparation of Compounds of Formula 209

[0081] Referring to Reaction Scheme 2, Step 4, the amine protecting group is then removed. When the protecting group is CBZ, this may be accomplished by treating a solution of a compound of Formula 207 in acetic acid containing 30% HBr. The product, a compound of Formula 209, is used in the next step without further purification.

Preparation of Compounds of Formula 211

[0082] Referring to Reaction Scheme 2, Step 5, to a solution of a compound of Formula 209 is a nonpolar, aprotic solvent such as dichloromethane at about 0°C is added sodium triacetoxyborohydride and an excess (preferably about 1.4 equivalents) of an aldehyde comprising $R_{7'}$ (i.e., a compound having the formula $R_{7'}CHO$ where $R_{7'}CH_2$- is equivalent to R_7 and R_7 is as described above or is a protected precursor to such a substituent, e.g., (3-oxo-propyl)-carbamic acid *tert*-butyl ester). The resulting mixture is stirred under nitrogen for about 2 h. Additional aldehyde and sodium triacetoxyborohydride are added. Stirring is continued for an additional 1 h. The product, a compound of Formula 211, is isolated and used in the next step without purification.

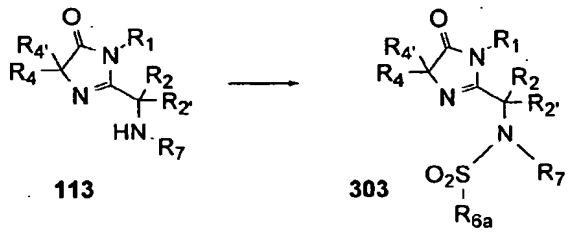
Preparation of Compounds of Formula 213

[0083] Referring to Reaction Scheme 2, Step 6, to a solution of a compound of Formula 211 in a nonpolar, aprotic solvent such as dichloromethane at about 0°C are

added a base such as DIEA and an excess (preferably about 1.1 equivalents) of an acid chloride of Formula R_6-(CO)-Cl. The resulting solution is stirred under nitrogen at room temperature for about 14 hours. The product, a compound of Formula 213, is isolated and purified.

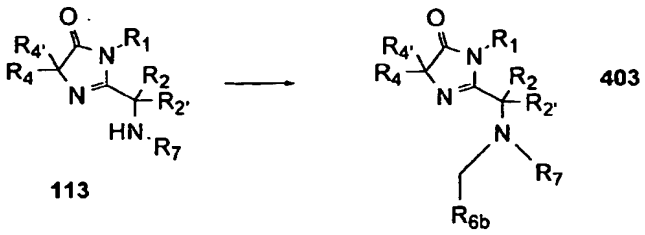
[0084] In an embodiment wherein R_7 further comprises a protected amine, the protecting group may be removed. For example, when the amino protecting group is Boc, this may be accomplished by treating a solution of a compound of Formula 213 in a nonpolar, aprotic solvent such as CH_2Cl_2 with trifluoroacetic acid. The product, the corresponding free amine, is isolated and purified.

Reaction Scheme 3



[0085] Referring to Reaction Scheme 3, to a solution of a compound of Formula 113 and an amine base such as diisopropylethylamine in a nonpolar, aprotic solvent such as dichloromethane is added a compound having the formula Cl-$S(O)_2$-R_{6a} or O-$(S(O)_2$-$R_{6a})_2$ where R_{6a} is as described above. The resulting solution is stirred under nitrogen at room temperature for several hours. The product, a compound of Formula 303, is isolated and purified.

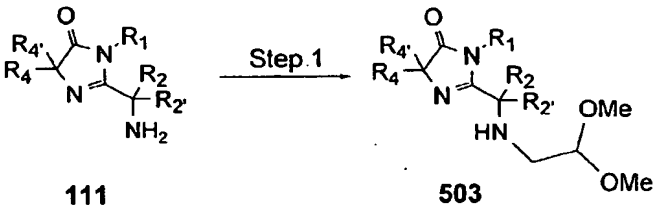
Reaction Scheme 4



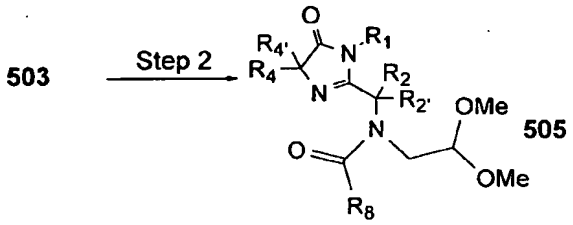
113 403

[0086] Referring to Reaction Scheme 4, to a solution of a compound of Formula 113 and an amine base such as diisopropylethylamine in a nonpolar, aprotic solvent such as dichloromethane is added a compound having the formula Cl-CH_2-R_{6b} where R_{6b} is as described above. The resulting solution is stirred under nitrogen at room temperature or with heat for several hours. The product, a compound of Formula 403, is isolated and purified.

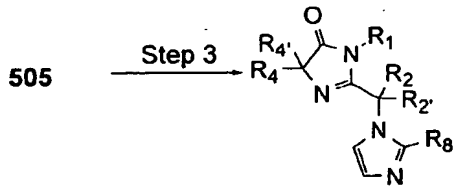
Reaction Scheme 5



111 503



503 505



505 507

Preparation of Formula 503

[0087] Referring to Reaction Scheme 5, Step 1, to an optionally substituted compound of Formula 111 dissolved in a polar, aprotic solvent (such as DMF) in the presence of a base (such as potassium carbonate) is added one equivalent of an optionally substituted suitably protected aldehyde wherein such aldehyde further comprises a leaving group, preferably, a halide (such as bromoacetaldehyde dimethylacetal). The solution is heated at reflux, monitoring completion of the reaction (e.g., by TLC). The reaction mixture is cooled and the corresponding, optionally substituted compound of Formula 503 is isolated and purified.

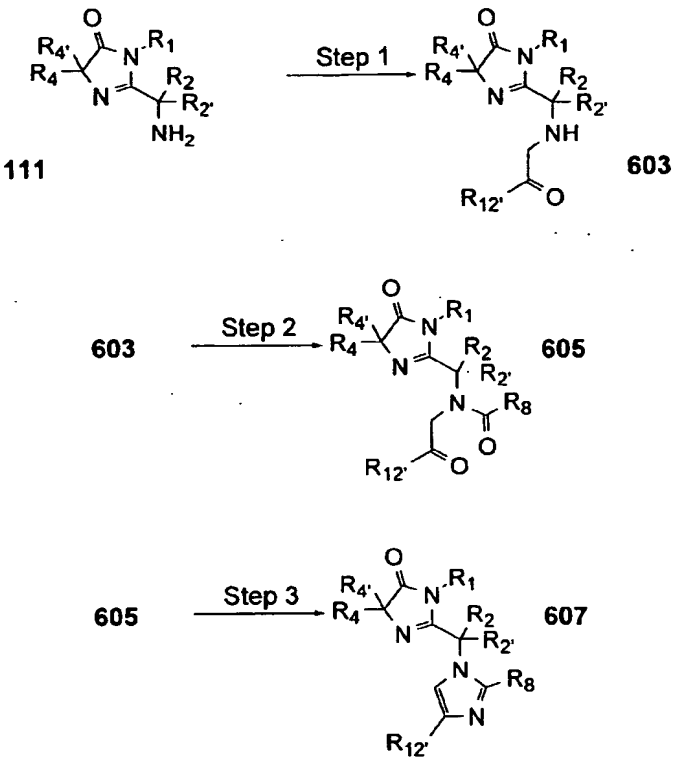
Preparation of Formula 505

[0088] Referring to Reaction Scheme 5, Step 2, to an optionally substituted compound of Formula 503 in an inert solvent (such as dichloromethane) in the presence of about 1.5 molar equivalents of an amine base (such as triethylamine) is added about 1.5 molar equivalents of an R_8 acid chloride, such as, Cl-C(O)-R_8, where R_8 is as described herein. The reaction takes place, with stirring, at room temperature over a period of 4 to 24 hours. Completion is monitored, e.g., by TLC. The corresponding compound of Formula 505 is isolated and purified.

Preparation of Formula 507

[0089] Referring to Reaction Scheme 5, Step 3, a solution of a compound of Formula 505 and an excess of ammonium acetate in acetic acid is heated at reflux for 1-4 hours. Completion is monitored, e.g., by TLC. The corresponding compound of Formula 507 is isolated and purified.

Reaction Scheme 6



111 603 603 605 605 607

Preparation of Formula 603

[0090] Referring to Reaction Scheme 6, Step 1, a suspension of a compound of Formula 111, an alpha-haloketone reagent of the Formula $R_{12'}(CO)CH_2Y$ wherein Y is a leaving group (preferably, a halide) and $R_{12'}$ is as described herein, and about an equivalent of a base, such as potassium carbonate in a polar, aprotic solvent such as DMF is stirred at room temperature. The reaction is diluted with water and the resulting solid, a compound of Formula 603, is used in the subsequent step without further purification.

Preparation of Formula 605

[0091] Referring to Reaction Scheme 6, Step 2, a solution of the compound of Formula 603, about an equivalent of an amine base, such as triethylamine and about an equivalent of an acid chloride (such as a compound of Formula R_8-COCl) in an organic solvent such as methylene chloride is stirred at room temperature for several hours. Completion is monitored, e.g., by TLC. The corresponding compound of Formula 605 is

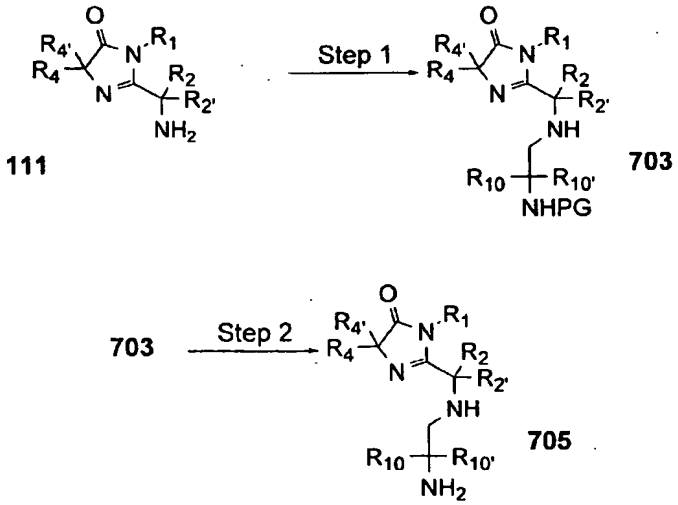
isolated and purified.

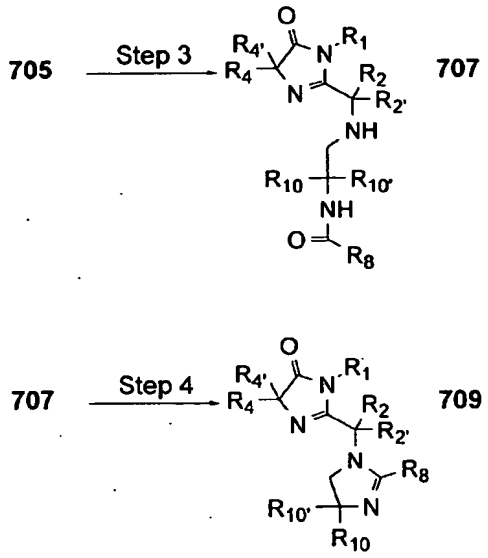
Preparation of Formula 607

[0092] Referring to Reaction Scheme 6, Step 3, a solution of a compound of Formula 605 and an excess of ammonium acetate in acetic acid is heated at reflux using a Dean-Stark trap and condenser. Completion is monitored, e.g., by TLC. The corresponding compound of Formula 607 is isolated and purified.

[0093] In an embodiment when $R_{12'}$ comprises a protected aminoalkyl group, the amino protecting group may be removed. For example, when the amino group is protected as the corresponding phthalimide, a solution of a compound of Formula 607 and an excess of anhydrous hydrazine in a polar, protic solvent such as ethanol is heated at reflux. The reaction is cooled to about 5°C and any precipitate is filtered off. The filtrate is concentrated in vacuo and purified to yield the corresponding free amine.

Reaction Scheme 7





Preparation of Formula 703

[0094] Referring to Reaction Scheme 7, Step 1, reductive amination of amines of Formula 111 with an optionally substituted, aldehyde-containing carbamic acid ester gives urethane intermediates. Removal of the Boc protecting group furnishes an amine of Formula 703.

[0095] More specifically, to a solution of a compound of Formula 111 and an equivalent of a suitably protected aldehyde (Seki *et. al. Chem. Pharm. Bull.* 1996, *44*, 2061) in dichloromethane is added a slight excess of a reducing agent, such as sodium triacetoxyborohydride. The resultant cloudy mixture is maintained at ambient temperature. Completion is monitored, e.g., by TLC. The corresponding compound of Formula 703 is isolated and used in the subsequent step without purification.

Preparation of Formula 705

[0096] Referring to Reaction Scheme 7, Step 2, to a solution of a compound of Formula 703 in a nonpolar, aprotic solvent such as dichloromethane is added a strong acid such as trifluoroacetic acid. The resultant solution is maintained at ambient temperature overnight and concentrated under reduced pressure. The residue is isolated

to give a compound of Formula 705 which is used in the subsequent step without purification.

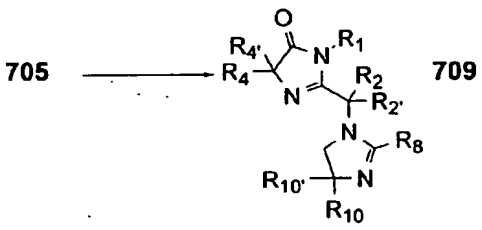
Preparation of Formula 707

[0097] Referring to Reaction Scheme 7, Step 3, to a solution of a compound of Formula 705 in a nonpolar, aprotic solvent such as dichloromethane is added an excess, preferably about two equivalents of an amine base such as triethylamine, followed by about an equivalent or slight excess of an acid chloride. The resultant solution is stirred at ambient temperature for about 3 hours. Completion is monitored, e.g., by TLC. The corresponding compound of Formula 707 is isolated and purified.

Preparation of Formula 709

[0098] Referring to Reaction Scheme 7, Step 4, a solution of a compound of Formula 707 in an excess of phosphorus oxychloride is heated at reflux. After 8 hours, the reaction mixture is allowed to cool to ambient temperature and concentrated under reduced pressure. The corresponding compound of Formula 709 is isolated and purified.

Reaction Scheme 8

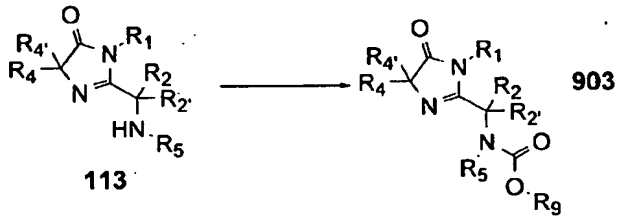


Preparation of Formula 709

[0099] As an alternative to Steps 3 and 4 of Reaction Scheme 7, acylation of primary amines of Formula 705, followed by acetic acid mediated cyclization, can proceed without isolation of the intermediate amides to provide the target compound of Formula 709. This route is shown in Reaction Scheme 8.

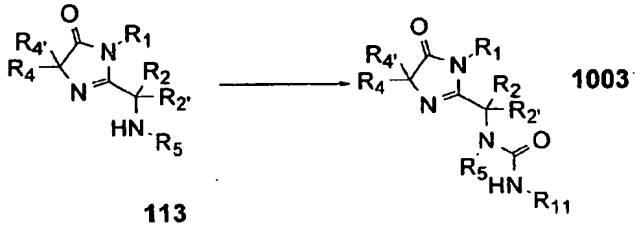
[00100] More specifically, to a solution of a compound of Formula 705 in a nonpolar, aprotic solvent such as dichloromethane is added an excess, preferably about two equivalents of an amine base, such as triethylamine, followed by about an equivalent of an acid chloride. The resultant solution is stirred at ambient temperature for 2 hours, then evaporated under reduced pressure. The resultant solid is treated with glacial acetic acid, then the resultant suspension is heated at reflux for about 48 hours. The reaction is cooled to ambient temperature then evaporated under reduced pressure. The corresponding compound of Formula 709 is isolated and purified.

Reaction Scheme 9



[00101] Referring to Reaction Scheme 9, a compound of Formula 113 is reacted with a slight excess of a compound of the formula $R_9O(CO)Cl$ in the presence of a base such as triethylamine in a nonpolar, aprotic solvent such as dichloromethane. The product, a compound of Formula 903 is isolated and purified.

Reaction Scheme 10



[00102] Referring to Reaction Scheme 10, a compound of Formula 113 is treated

with a slight excess of an isocyanate R_{11}-N=C=O in the presence of a base, such as triethylamine, in a nonpolar, aprotic solvent, such as dichloromethane. The product, a compound of Formula 1003, is isolated and purified.

Particular Processes and Last Steps

[00103] A compound of Formula I is optionally contacted with a pharmaceutically acceptable acid or base to form the corresponding acid or base addition salt.

[00104] A pharmaceutically acceptable acid addition salt of a compound of Formula I is optionally contacted with a base to form the corresponding free base of Formula I. A pharmaceutically acceptable base addition salt of a compound of Formula I is optionally contacted with an acid to form the corresponding free acid of Formula I.

[00105] A racemic mixture of isomers of a compound of Formula I is placed on a chromatography column and separated into (R)- and (S)- enantiomers.

Particular Embodiments of Compounds of the Invention

R_1

[00106] When considering the compounds of Formula I, in a particular embodiment R_1 is selected from hydrogen, optionally substituted alkyl, optionally substituted aryl, optionally substituted heteroaryl, optionally substituted aralkyl, and optionally substituted heteroaralkyl (especially optionally substituted aryl and optionally substituted aryl-C_1-C_4-alkyl-). In a more particular embodiment R_1 is selected from hydrogen, optionally substituted lower alkyl, optionally substituted benzyl, optionally substituted naphthalenylmethyl, optionally substituted phenyl, and naphthyl. More particularly, R_1 is optionally substituted phenyl-C_1-C_4-alkyl- or optionally substituted heteroaryl-C_1-C_4-alkyl-.

[00107] In a particular embodiment R_1 is chosen from hydrogen, naphthyl, phenyl, bromophenyl, chlorophenyl, methoxyphenyl, ethoxyphenyl, tolyl, dimethylphenyl,

chorofluorophenyl, methylchlorophenyl, ethylphenyl, phenethyl, benzyl, chlorobenzyl, methylbenzyl, methoxybenzyl, cyanobenzyl, hydroxybenzyl, dichlorobenzyl, dimethoxybenzyl, or naphthalenylmethyl. More particularly, R_1 is benzyl, cyanobenzyl, methoxybenzyl, or naphthalenylmethyl. Most particularly, R_1 is benzyl.

R_2

[00108] As will be appreciated by those skilled in the art, the compounds described herein possess a potentially chiral center at the carbon to which R_2 is attached. The R_2 and R_2' groups may be the same or different; if different, the composition is chiral. When the R_2 and R_2' are different, preferred embodiments utilize only a single non-hydrogen R_2. The invention contemplates the use of pure enantiomers and mixtures of enantiomers, including racemic mixtures, although the use of a substantially optically pure enantiomer will generally be preferred. The term "substantially pure" means having at least about 95% chemical purity with no single impurity greater than about 1%. The term "substantially optically pure" or "enantiomerically pure" means having at least about 90% enantiomeric excess. In a particular embodiment, the stereogenic center to which R_2 and R_2' are attached is of the R configuration.

[00109] In a particular embodiment, R_2 is optionally substituted alkyl, and R_2' is hydrogen. In a more particular embodiment R_2 is chosen from methyl, ethyl, propyl (especially, c-propyl or i-propyl), butyl (especially, t-butyl), methylthioethyl, methylthiomethyl, aminobutyl, (CBZ)aminobutyl, cyclohexylmethyl, benzyloxymethyl, methylsulfinylethyl, methylsulfinylmethyl, hydroxymethyl, phenyl, benzyl and indolylmethyl. A particular embodiment is drawn to the R enantiomer where R_2 is i-propyl.

R_4 and R_4'

[00110] When considering the compounds of Formula I, in one embodiment R_4 and R_4' are independently chosen from hydrogen, optionally substituted alkyl, optionally substituted aryl, optionally substituted aralkyl, optionally substituted heteroaryl, and optionally substituted heteroaralkyl. Particularly, R_4 and R_4' are independently chosen

from hydrogen, optionally substituted aryl and optionally substituted aryl-C_1-C_4-alkyl-.

[00111] In another embodiment, R_4 and $R_{4'}$, together with the carbon to which they are attached form an optionally substituted 3- to 7-membered ring. More particularly, R_4 and $R_{4'}$, together with the carbon to which they are attached form a cyclopropyl ring.

[00112] In another embodiment, R_4 is an optionally substituted alkylidene of the formula $=C(R_5)(R_{5'})$ and $R_{4'}$ is absent wherein R_5 and $R_{5'}$ are independently selected from hydrogen, optionally substituted alkyl, and optionally substituted aryl. More particularly, R_5 and $R_{5'}$ are independently lower-alkyl. In another particular embodiment, R_4 is an isopropylidene group and $R_{4'}$ is absent.

R_6 Groups When R_3 is $-N(R_7)(COR_6)$

[00113] When considering the compounds of Formula I wherein R_3 is $-N(R_7)(COR_6)$, in one embodiment R_6 is selected from hydrogen, optionally substituted alkyl, optionally substituted aralkyl-, optionally substituted heteroaralkyl-, optionally substituted heteroaryl, optionally substituted aryl, R_9O- and R_{11}-NH-, R_9 is chosen from optionally substituted alkyl and optionally substituted aryl, and R_{11} is chosen from hydrogen, optionally substituted alkyl and optionally substituted aryl. Particular R_6 are optionally substituted alkyl (e.g., C_1-C_8 alkyl substituted with lower-alkoxy), optionally substituted heteroaryl, and optionally substituted aryl.

[00114] In a more particular embodiment, when R_6 is not $R_{11}NH$- or R_9O-, R_6 is chosen from phenyl; phenyl substituted with one or more of the following substituents: halo, alkyl, alkyl substituted with hydroxy (e.g., hydroxymethyl), alkoxy, alkyl substituted with alkoxy, nitro, formyl, carboxy, cyano, methylenedioxy, ethylenedioxy, acyl (e.g., acetyl), -N-acyl (e.g., N-acetyl) or trifluoromethyl; benzyl; phenoxymethyl-; halophenoxymethyl-; phenylvinyl-; heteroaryl-; heteroaryl- substituted with alkyl or alkyl substituted with halo (e.g., CF_3); alkyl substituted with alkoxy- and benzyloxymethyl-.

[00115] In a most particular embodiment, when R_6 is not $R_{11}NH$- or R_9O-, R_6 is chosen from phenyl, halophenyl, dihalophenyl, cyanophenyl, halo(trifluoromethyl)phenyl, hydroxymethylphenyl, methoxyphenyl, ethoxyphenyl, carboxyphenyl, ethylphenyl, tolyl, methylenedioxyphenyl, ethlenedixoyphenyl, methoxychlorophenyl, dihydro-

benzodioxinyl, methylhalophenyl, trifluoromethylphenyl, bis(trifluoromethyl)phenylbenzyl, furanyl, alkyl substituted furanyl, trifluoromethylfuranyl, alkyl substituted trifluoromethylfuranyl, benzofuranyl, thiophenyl, alkyl substituted thiophenyl, benzothiophenyl, benzothiadiazolyl, pyridinyl, indolyl, methylpyridinyl, trifluoromethylpyridinyl, pyrrolyl, quinolinyl, picolinyl, pyrazolyl, alkyl substituted pyrazolyl, N-methyl pyrazolyl, alkyl substituted N-methyl pyrazolyl, alkyl substituted pyrazinyl, alkyl substituted isoxazolyl, benzoisoxazolyl, morpholinomethyl, methylthiomethyl, methoxymethyl, N-methyl imidazolyl, and imidazolyl. Yet more preferably, R_6 is tolyl, halophenyl, halomethylphenyl, hydroxymethylphenyl, methylenedioxyphenyl, formylphenyl or cyanophenyl.

[00116] In a more particular embodiment, when R_6 is $R_{11}NH$-, R_{11} is chosen from hydrogen, lower-alkyl; cyclohexyl; phenyl; and phenyl substituted with halo, lower-alkyl, lower-alkoxy, or lower-alkylthio.

[00117] In a most particular embodiment, when R_6 is $R_{11}NH$-, R_{11} is hydrogen isopropyl, butyl, cyclohexyl, phenyl, bromophenyl, dichlorophenyl, methoxyphenyl, ethylphenyl, tolyl, trifluoromethylphenyl or methylthiophenyl.

[00118] In an embodiment, wherein R_6 is R_9O-, R_9 is chosen from optionally substituted alkyl and optionally substituted aryl.

R_{6a}Groups when R_3 is $-N(R_7)(SO_2R_{6a})$

[00119] In one embodiment, when R_3 is $-N(R_7)(SO_2R_{6a})$, R_{6a} is chosen from alkyl; phenyl; naphthyl; phenyl substituted with halo, lower-alkyl, lower-alkoxy, cyano, nitro, methylenedioxy, or trifluoromethyl; biphenylyl and heteroaryl. More particularly, R_{6a} is chosen from phenyl substituted with halo, lower-alkyl, lower-alkoxy, cyano, nitro, methylenedioxy, or trifluoromethyl and naphthyl.

R_{6b} Groups when R_3 is $-N(R_7)(CH_2R_{6b})$

[00120] In one embodiment, when R_3 is $-N(R_7)(CH_2R_{6b})$, R_{6b} is chosen from alkyl; substituted lower-alkyl; phenyl; naphthyl; phenyl substituted with carboxy, alkoxycarbonyl cyano, halo, lower-alkyl-, lower-alkoxy, nitro, methylenedioxy, or

trifluoromethyl; biphenylyl, benzyl; and heterocyclyl.

[00121] More particularly, R_{6b} is chosen from halophenyl, polyhalophenyl, methylhalophenyl, tolyl, dimethylphenyl, methoxyphenyl, dimethoxyphenyl, cyanophenyl, trifluoromethylphenyl, trifluoromethoxyphenyl, bis(trifluoromethyl)phenyl, carboxyphenyl, t-butylphenyl, methoxycarbonylphenyl, piperidinyl and naphthyl.

R_7 Groups when R_3 is $-NHR_7$, $-N(R_7)(COR_6)$, or $-N(R_7)(CH_2R_{6b})$

[00122] In one embodiment when R_3 is $-NHR_7$, $-N(R_7)(COR_6)$, or $-N(R_7)(CH_2R_{6b})$, R_7 is chosen from hydrogen, optionally substituted alkyl, optionally substituted aryl, optionally substituted aralkyl-, optionally substituted heterocyclyl, and optionally substituted heteroaralkyl- (preferably hydrogen or optionally substituted alkyl).

[00123] More particularly, R_7 is chosen from lower-alkyl; cyclohexyl; phenyl substituted with hydroxy, lower-alkoxy or lower-alkyl; benzyl; heteroarylmethyl-; heteroarylethyl-; heteroarylpropyl-; and R_{14}-alkylene-, wherein R_{14} is hydroxy, di(lower-alkyl)amino-, (lower-alkyl)amino-, amino, lower-alkoxy-, or N-heterocyclyl-, particularly pyrrolidino, piperidino or imidazolyl.

[00124] Yet more particularly, R_7 is R_{14}-alkylene-, wherein R_{14} is amino, lower-alkylamino-, di(lower-alkyl)amino-, lower-alkoxy-, hydroxyl, or N-heterocyclyl. Most particularly R_{14} is amino.

[00125] In a most particular embodiment when R_3 is $-NHR_7$, $-N(R_7)(COR_6)$, or $-N(R_7)(CH_2R_{6b})$, R_7 is chosen from hydrogen, methyl, ethyl, propyl, butyl, cyclohexyl, carboxyethyl, carboxymethyl, methoxyethyl, hydroxyethyl, hydroxypropyl, dimethylaminoethyl, dimethylaminopropyl, diethylaminoethyl, diethylaminopropyl, aminopropyl, methylaminopropyl, 2,2-dimethyl-3-(dimethylamino)propyl, 1-cyclohexyl-4-(diethylamino)butyl, aminoethyl, aminobutyl, aminopentyl, aminohexyl, aminoethoxyethyl, isopropylaminopropyl, diisopropylaminoethyl, 1-methyl-4-(diethylamino)butyl, (t-Boc)aminopropyl, hydroxyphenyl, benzyl, methoxyphenyl, methylmethoxyphenyl, dimethylphenyl, tolyl, ethylphenyl, (oxopyrrolidinyl)propyl, (methoxycarbonyl)ethyl, benzylpiperidinyl, pyridinylethyl, pyridinylmethyl, morpholinylethyl, morpholinylpropyl, piperidinyl, azetidinylmethyl, azetidinylethyl,

azetidinylpropyl, pyrrolidinylethyl, pyrrolidinylpropyl, piperidinylmethyl, piperidinylethyl, imidazolylpropyl, imidazolylethyl, (ethylpyrrolidinyl)methyl, (methylpyrrolidinyl)ethyl, (methylpiperidinyl)propyl, (methylpiperazinyl)propyl, furanylmethyl and indolylethyl.

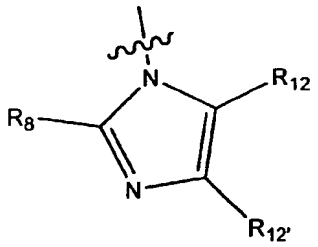
[00126] More particularly, R_7 is aminoethyl, aminopropyl, aminobutyl, aminopentyl, aminohexyl, methylaminoethyl, methylaminopropyl, methylaminobutyl, methylaminopentyl, methylaminohexyl, dimethylaminoethyl, dimethylaminopropyl, dimethylaminobutyl, dimethylaminopentyl, dimethylaminohexyl, ethylaminoethyl, ethylaminopropyl, ethylaminobutyl, ethylaminopentyl, ethylaminohexyl, diethylaminoethyl, diethylaminopropyl, diethylaminobutyyl, diethylaminopentyl, or diethylaminohexyl, most preferably aminopropyl.

R_7 Groups when R_3 is $-N(R_7)(SO_2R_{6a})$

[00127] In one embodiment, when R_3 is $-N(R_7)(SO_2R_{6a})$, R_7 is chosen from lower-alkyl, cyclohexyl; phenyl substituted with hydroxy, lower-alkoxy or lower-alkyl; benzyl; heteroarylmethyl-; heteroarylethyl-; heteroarylpropyl-; and R_{14}-alkylene-, wherein R_{14} is hydroxy, di(lower-alkyl)amino-, (lower-alkyl)amino-, amino, lower-alkoxy-, or N-heterocyclyl-, particularly pyrrolidino, piperidino or imidazolyl.

R_3 is an Imidazole

[00128] In one embodiment, when R_3 is an imidazole, R_3 has the formula:



wherein

R_8 is chosen from hydrogen, optionally substituted alkyl, optionally substituted

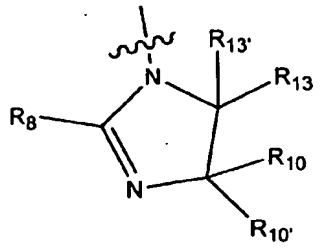
aryl, optionally substituted aralkyl -, optionally substituted heteroaralkyl -, optionally substituted aralkoxy -, optionally substituted heteroaralkoxy -, optionally substituted heteroaryl-; and

R_{12} and $R_{12'}$ are independently hydrogen, optionally substituted alkyl, optionally substituted aryl, or optionally substituted aralkyl - (preferably optionally substituted alkyl). More particularly, R_8 is phenyl substituted with lower-alkyl, lower-alkoxy-, and/or halo (especially lower-alkyl and/or halo); phenyl; or benzyl. In one embodiment, R_8 is phenyl substituted with halo and/or methyl.

[00129] In a particular embodiment, R_{12} is hydrogen and $R_{12'}$ is substituted C_1-C_4 alkyl. More particularly, R_{12} is hydrogen and $R_{12'}$ is aminomethyl, aminoethyl, aminopropyl, acetylamino-methyl, acetylaminoethyl, benzyloxycarbonylaminomethyl or benzyloxycarbonylaminoethyl.

R_3 is an Imidazoline

[00130] In one embodiment, when R_3 is an imidazoline, R_3 has the formula



wherein R_8 is chosen from hydrogen, optionally substituted alkyl, optionally substituted aryl, optionally substituted aralkyl-, and optionally substituted heteroaryl-; and R_{10}, $R_{10'}$, R_{13}, and $R_{13'}$ are independently chosen from hydrogen, optionally substituted alkyl, optionally substituted aryl, and optionally substituted aralkyl-.

[00131] More particularly, R_8 is methylenedioxyphenyl; phenyl; phenyl substituted with lower-alkyl, lower-alkoxy, and/or halo; or benzyl. In a particular embodiment, R_8 is methylenedioxyphenyl-; phenyl; or phenyl substituted with methoxy, halo and/or methyl (preferably halo and/or methyl, including tolyl); more preferably methylenedioxyphenyl

or said substituted phenyls.

[00132] In another particular embodiment, R_{10}, $R_{10'}$, $R_{13'}$, and R_{13} are independently hydrogen or optionally substituted alkyl (preferably optionally substituted lower-alkyl). More particularly, R_{10} and $R_{10'}$ are independently selected from the group consisting of hydrogen or optionally substituted C_1-C_4 alkyl (and more particularly, methyl or aminoalkyl-) and $R_{13'}$ and R_{13} are hydrogen.

[00133] Particular compounds are:

N-(3-Amino-propyl)-N-[1-(6-benzyl-7-oxo-4,6-diaza-spiro[2.4]hept-4-en-5-yl)-2-methyl-propyl]-4-methyl-benzamide; and

N-(3-Amino-propyl)-N-[1-(1-benzyl-4-isopropylidene-5-oxo-4,5-dihydro-1H-imidazol-2-yl)-2-methyl-propyl]-4-methyl-benzamide

Utility, Testing and Administration

General Utility

[00134] Once made, the compounds of the invention find use in a variety of applications involving alteration of mitosis. As will be appreciated by those skilled in the art, mitosis may be altered in a variety of ways; that is, one can affect mitosis either by increasing or decreasing the activity of a component in the mitotic pathway. Stated differently, mitosis may be affected (e.g., disrupted) by disturbing equilibrium, either by inhibiting or activating certain components. Similar approaches may be used to alter meiosis.

[00135] In one embodiment, the compounds of the invention are used to inhibit mitotic spindle formation, thus causing prolonged cell cycle arrest in mitosis. By "inhibit" in this context is meant decreasing or interfering with mitotic spindle formation or causing mitotic spindle dysfunction. By "mitotic spindle formation" herein is meant organization of microtubules into bipolar structures by mitotic kinesins. By "mitotic spindle dysfunction" herein is meant mitotic arrest and monopolar spindle formation.

[00136] The compounds of the invention are useful to bind to, and/or inhibit the activity of, a mitotic kinesin, KSP. In one embodiment, the KSP is human KSP, although

the compounds may be used to bind to or inhibit the activity of KSP kinesins from other organisms. In this context, "inhibit" means either increasing or decreasing spindle pole separation, causing malformation, i.e., splaying, of mitotic spindle poles, or otherwise causing morphological perturbation of the mitotic spindle. Also included within the definition of KSP for these purposes are variants and/or fragments of KSP. See U.S. Patent 6,437,115, hereby incorporated by reference in its entirety. The compounds of the invention have been shown to have specificity for KSP. However, the present invention includes the use of the compounds to bind to or modulate other mitotic kinesins.

[00137] The compounds of the invention are used to treat cellular proliferation diseases. Such disease states which can be treated by the compounds, compositions and methods provided herein include, but are not limited to, cancer (further discussed below), autoimmune disease, fungal disorders, arthritis, graft rejection, inflammatory bowel disease, cellular proliferation induced after medical procedures, including, but not limited to, surgery, angioplasty, and the like. Treatment includes inhibiting cellular proliferation. It is appreciated that in some cases the cells may not be in an abnormal state and still require treatment. Thus, in one embodiment, the invention herein includes application to cells or individuals afflicted or subject to impending affliction with any one of these disorders or states.

[00138] The compounds, compositions and methods provided herein are particularly deemed useful for the treatment of cancer including solid tumors such as skin, breast, brain, cervical carcinomas, testicular carcinomas, etc. More particularly, cancers that may be treated by the compounds, compositions and methods of the invention include, but are not limited to: Cardiac: sarcoma (angiosarcoma, fibrosarcoma, rhabdomyosarcoma, liposarcoma), myxoma, rhabdomyoma, fibroma, lipoma and teratoma; Lung: bronchogenic carcinoma (squamous cell, undifferentiated small cell, undifferentiated large cell, adenocarcinoma), alveolar (bronchiolar) carcinoma, bronchial adenoma, sarcoma, lymphoma, chondromatous hamartoma, mesothelioma; Gastrointestinal: esophagus (squamous cell carcinoma, adenocarcinoma, leiomyosarcoma, lymphoma), stomach (carcinoma, lymphoma, leiomyosarcoma), pancreas (ductal adenocarcinoma, insulinoma, glucagonoma, gastrinoma, carcinoid

tumors, vipoma), small bowel (adenocarcinoma, lymphoma, carcinoid tumors, Karposi's sarcoma, leiomyoma, hemangioma, lipoma, neurofibroma, fibroma), large bowel (adenocarcinoma, tubular adenoma, villous adenoma, hamartoma, leiomyoma); Genitourinary tract: kidney (adenocarcinoma, Wilm's tumor (nephroblastoma), lymphoma, leukemia), bladder and urethra (squamous cell carcinoma, transitional cell carcinoma, adenocarcinoma), prostate (adenocarcinoma, sarcoma), testis (seminoma, teratoma, embryonal carcinoma, teratocarcinoma, choriocarcinoma, sarcoma, interstitial cell carcinoma, fibroma, fibroadenoma, adenomatoid tumors, lipoma); Liver: hepatoma (hepatocellular carcinoma), cholangiocarcinoma, hepatoblastoma, angiosarcoma, hepatocellular adenoma, hemangioma; Bone: osteogenic sarcoma (osteosarcoma), fibrosarcoma, malignant fibrous histiocytoma, chondrosarcoma, Ewing's sarcoma, malignant lymphoma (reticulum cell sarcoma), multiple myeloma, malignant giant cell tumor chordoma, osteochronfroma (osteocartilaginous exostoses), benign chondroma, chondroblastoma, chondromyxofibroma, osteoid osteoma and giant cell tumors; Nervous system: skull (osteoma, hemangioma, granuloma, xanthoma, osteitis deformans), meninges (meningioma, meningiosarcoma, gliomatosis), brain (astrocytoma, medulloblastoma, glioma, ependymoma, germinoma (pinealoma), glioblastoma multiform, oligodendroglioma, schwannoma, retinoblastoma, congenital tumors), spinal cord neurofibroma, meningioma, glioma, sarcoma); Gynecological: uterus (endometrial carcinoma), cervix (cervical carcinoma, pre-tumor cervical dysplasia), ovaries (ovarian carcinoma (serous cystadenocarcinoma, mucinous cystadenocarcinoma, unclassified carcinoma), granulosa-thecal cell tumors, Sertoli-Leydig cell tumors, dysgerminoma, malignant teratoma), vulva (squamous cell carcinoma, intraepithelial carcinoma, adenocarcinoma, fibrosarcoma, melanoma), vagina (clear cell carcinoma, squamous cell carcinoma, botryoid sarcoma (embryonal rhabdomyosarcoma), fallopian tubes (carcinoma); Hematologic: blood (myeloid leukemia (acute and chronic), acute lymphoblastic leukemia, chronic lymphocytic leukemia, myeloproliferative diseases, multiple myeloma, myelodysplastic syndrome), Hodgkin's disease, non-Hodgkin's lymphoma (malignant lymphoma); Skin: malignant melanoma, basal cell carcinoma, squamous cell carcinoma, Karposi's sarcoma, moles dysplastic nevi, lipoma, angioma,

dermatofibroma, keloids, psoriasis; and Adrenal glands: neuroblastoma. Thus, the term "cancerous cell" as provided herein, includes a cell afflicted by any one of the above identified conditions.

Testing

[00139] For assay of KSP-modulating activity, generally either KSP or a compound according to the invention is non-diffusably bound to an insoluble support having isolated sample receiving areas (e.g., a microtiter plate, an array, etc.). The insoluble support may be made of any composition to which the sample can be bound, is readily separated from soluble material, and is otherwise compatible with the overall method of screening. The surface of such supports may be solid or porous and of any convenient shape. Examples of suitable insoluble supports include microtiter plates, arrays, membranes and beads. These are typically made of glass, plastic (e.g., polystyrene), polysaccharides, nylon or nitrocellulose, Teflon™, etc. Microtiter plates and arrays are especially convenient because a large number of assays can be carried out simultaneously, using small amounts of reagents and samples. The particular manner of binding of the sample is not crucial so long as it is compatible with the reagents and overall methods of the invention, maintains the activity of the sample and is nondiffusable. Preferred methods of binding include the use of antibodies (which do not sterically block either the ligand binding site or activation sequence when the protein is bound to the support), direct binding to "sticky" or ionic supports, chemical crosslinking, the synthesis of the protein or agent on the surface, etc. Following binding of the sample, excess unbound material is removed by washing. The sample receiving areas may then be blocked through incubation with bovine serum albumin (BSA), casein or other innocuous protein or other moiety.

[00140] The compounds of the invention may be used on their own to inhibit the activity of a mitotic kinesin, particularly KSP. In one embodiment, a compound of the invention is combined with KSP and the activity of KSP is assayed. Kinesin (including KSP) activity is known in the art and includes one or more kinesin activities. Kinesin activities include the ability to affect ATP hydrolysis; microtubule binding; gliding and

polymerization/depolymerization (effects on microtubule dynamics); binding to other proteins of the spindle; binding to proteins involved in cell-cycle control; serving as a substrate to other enzymes, such as kinases or proteases; and specific kinesin cellular activities such as spindle pole separation.

[00141] Methods of performing motility assays are well known to those of skill in the art. (See e.g., Hall, et al. (1996), Biophys. J., 71: 3467-3476, Turner et al., 1996, AnaL Biochem. 242 (1):20-5; Gittes et al., 1996, Biophys. J. 70(I): 418-29; Shirakawa et al., 1995, J. Exp. BioL 198: 1809-15; Winkelmann et al., 1995, Biophys. J. 68: 2444-53; Winkelmann et al., 1995, Biophys. J. 68: 72S.)

[00142] Methods known in the art for determining ATPase hydrolysis activity also can be used. Suitably, solution based assays are utilized. U.S. Patent 6,410,254, hereby incorporated by reference in its entirety, describes such assays. Alternatively, conventional methods are used. For example, P_i release from kinesin can be quantified. In one particular embodiment, the ATPase hydrolysis activity assay utilizes 0.3 M PCA (perchloric acid) and malachite green reagent (8.27 mM sodium molybdate II, 0.33 mM malachite green oxalate, and 0.8 mM Triton X-1 00). To perform the assay, 10 μL of the reaction mixture is quenched in 90 μL of cold 0.3 M PCA. Phosphate standards are used so data can be converted to mM inorganic phosphate released. When all reactions and standards have been quenched in PCA, 100 μL of malachite green reagent is added to the relevant wells in e.g., a microtiter plate. The mixture is developed for 10-15 minutes and the plate is read at an absorbance of 650 nm. If phosphate standards were used, absorbance readings can be converted to mM P_i and plotted over time. Additionally, ATPase assays known in the art include the luciferase assay.

[00143] ATPase activity of kinesin motor domains also can be used to monitor the effects of agents and are well known to those skilled in the art. In one embodiment ATPase assays of kinesin are performed in the absence of microtubules. In another embodiment, the ATPase assays are performed in the presence of microtubules. Different types of agents can be detected in the above assays. In one embodiment, the effect of a agent is independent of the concentration of microtubules and ATP. In another embodiment, the effect of the agents on kinesin ATPase can be decreased by increasing

the concentrations of ATP, microtubules or both. In yet another embodiment, the effect of the agent is increased by increasing concentrations of ATP, microtubules or both.

[00144] Compounds that inhibit the biochemical activity of KSP in vitro may then be screened in vivo. In vivo screening methods include assays of cell cycle distribution, cell viability, or the presence, morphology, activity, distribution, or number of mitotic spindles. Methods for monitoring cell cycle distribution of a cell population, for example, by flow cytometry, are well known to those skilled in the art, as are methods for determining cell viability. See for example, U.S. Patent 6,437,115, hereby incorporated by reference in its entirety. Microscopic methods for monitoring spindle formation and malformation are well known to those of skill in the art (see, e.g., Whitehead and Rattner (1998), J. Cell Sci. 111:2551-61; Galgio et al, (1996) J. Cell Biol., 135:399-414), each incorporated herein by reference in its entirety.

[00145] The compounds of the invention inhibit the KSP kinesin. One measure of inhibition is IC_{50}, defined as the concentration of the compound at which the activity of KSP is decreased by fifty percent relative to a control. Preferred compounds have IC_{50}'s of less than about 1 mM, with preferred embodiments having IC_{50}'s of less than about 100 μM, with more preferred embodiments having IC_{50}'s of less than about 10 μM, with particularly preferred embodiments having IC_{50}'s of less than about 1 μM, and especially preferred embodiments having IC_{50}'s of less than about 100 nM, and with the most preferred embodiments having IC_{50}'s of less than about 10 nM. Measurement of IC_{50} is done using an ATPase assay such as described herein.

[00146] Another measure of inhibition is K_i. For compounds with IC_{50}'s less than 1 μM, the K_i or K_d is defined as the dissociation rate constant for the interaction of the compounds described herein with KSP. Preferred compounds have K_i's of less than about 100 μM, with preferred embodiments having K_i's of less than about 10 μM, and particularly preferred embodiments having K_i's of less than about 1 μM and especially preferred embodiments having K_i's of less than about 100 nM, and with the most preferred embodiments having K_i's of less than about 10 nM.

[00147] The K_i for a compound is determined from the IC_{50} based on three assumptions and the Michaelis-Menten equation. First, only one compound molecule

binds to the enzyme and there is no cooperativity. Second, the concentrations of active enzyme and the compound tested are known (i.e., there are no significant amounts of impurities or inactive forms in the preparations). Third, the enzymatic rate of the enzyme-inhibitor complex is zero. The rate (i.e., compound concentration) data are fitted to the equation:

$$V = V_{max}E_0 \left[I - \frac{(E_0 + I_0 + Kd) - \sqrt{(E_0 + I_0 + Kd)^2 - 4E_0 I_0}}{2E_0} \right]$$

where V is the observed rate, V_{max} is the rate of the free enzyme, I_0 is the inhibitor concentration, E_0 is the enzyme concentration, and K_d is the dissociation constant of the enzyme-inhibitor complex.

[00148] Another measure of inhibition is GI_{50}, defined as the concentration of the compound that results in a decrease in the rate of cell growth by fifty percent. Preferred compounds have GI_{50}'s of less than about 1 mM; those having a GI_{50} of less than about 20 μM are more preferred; those having a GI_{50} of less than about 10 μM more so; those having a GI_{50} of less than about 1 μM more so; those having a GI_{50} of less than about 100 nM more so; and those having a GI_{50} of less than about 10 nM even more so. Measurement of GI_{50} is done using a cell proliferation assay such as described herein. Compounds of this class were found to inhibit cell proliferation.

[00149] In vitro potency of small molecule inhibitors is determined, for example, by assaying human ovarian cancer cells (SKOV3) for viability following a 72-hour exposure to a 9-point dilution series of compound. Cell viability is determined by measuring the absorbance of formazon, a product formed by the bioreduction of MTS/PMS, a commercially available reagent. Each point on the dose-response curve is calculated as a percent of untreated control cells at 72 hours minus background absorption (complete cell kill).

[00150] Anti-proliferative compounds that have been successfully applied in the clinic to treatment of cancer (cancer chemotherapeutics) have GI_{50}'s that vary greatly. For example, in A549 cells, paclitaxel GI_{50} is 4 nM, doxorubicin is 63 nM, 5-fluorouracil is 1 μM, and hydroxyurea is 500 μM (data provided by National Cancer Institute,

Developmental Therapeutic Program, http://dtp.nci.nih.gov/). Therefore, compounds that inhibit cellular proliferation, irrespective of the concentration demonstrating inhibition, may be useful.

[00151] To employ the compounds of the invention in a method of screening for compounds that bind to KSP kinesin, the KSP is bound to a support, and a compound of the invention is added to the assay. Alternatively, the compound of the invention is bound to the support and KSP is added. Classes of compounds among which novel binding agents may be sought include specific antibodies, non-natural binding agents identified in screens of chemical libraries, peptide analogs, etc. Of particular interest are screening assays for candidate agents that have a low toxicity for human cells. A wide variety of assays may be used for this purpose, including labeled in vitro protein-protein binding assays, electrophoretic mobility shift assays, immunoassays for protein binding, functional assays (phosphorylation assays, etc.) and the like.

[00152] The determination of the binding of the compound of the invention to KSP may be done in a number of ways. In a preferred embodiment, the compound is labeled, for example, with a fluorescent or radioactive moiety, and binding is determined directly. For example, this may be done by attaching all or a portion of KSP to a solid support, adding a labeled test compound (for example a compound of the invention in which at least one atom has been replaced by a detectable isotope), washing off excess reagent, and determining whether the amount of the label is that present on the solid support.

[00153] By "labeled" herein is meant that the compound is either directly or indirectly labeled with a label which provides a detectable signal, e.g., radioisotope, fluorescent tag, enzyme, antibodies, particles such as magnetic particles, chemiluminescent tag, or specific binding molecules, etc. Specific binding molecules include pairs, such as biotin and streptavidin, digoxin and antidigoxin etc. For the specific binding members, the complementary member would normally be labeled with a molecule which provides for detection, in accordance with known procedures, as outlined above. The label can directly or indirectly provide a detectable signal.

[00154] In some embodiments, only one of the components is labeled. For example, the kinesin proteins may be labeled at tyrosine positions using ^{125}I, or with

fluorophores. Alternatively, more than one component may be labeled with different labels; using ^{125}I for the proteins, for example, and a fluorophor for the antimitotic agents.

[00155] The compounds of the invention may also be used as competitors to screen for additional drug candidates. "Candidate agent" or "drug candidate" or grammatical equivalents as used herein describe any molecule, e.g., protein, oligopeptide, small organic molecule, polysaccharide, polynucleotide, etc., to be tested for bioactivity. They may be capable of directly or indirectly altering the cellular proliferation phenotype or the expression of a cellular proliferation sequence, including both nucleic acid sequences and protein sequences. In other cases, alteration of cellular proliferation protein binding and/or activity is screened. Screens of this sort may be performed either in the presence or absence of microtubules. In the case where protein binding or activity is screened, preferred embodiments exclude molecules already known to bind to that particular protein, for example, polymer structures such as microtubules, and energy sources such as ATP. Preferred embodiments of assays herein include candidate agents which do not bind the cellular proliferation protein in its endogenous native state termed herein as "exogenous" agents. In another preferred embodiment, exogenous agents further exclude antibodies to KSP.

[00156] Candidate agents can encompass numerous chemical classes, though typically they are small organic compounds having a molecular weight of more than 100 and less than about 2,500 daltons. Candidate agents comprise functional groups necessary for structural interaction with proteins, particularly hydrogen bonding and lipophilic binding, and typically include at least an amine, carbonyl, hydroxyl, ether, or carboxyl group, and often at least two of the functional chemical groups. The candidate agents often comprise cyclical carbon or heterocyclic structures and/or aromatic or polyaromatic structures substituted with one or more of the above functional groups. Candidate agents are also found among biomolecules including peptides, saccharides, fatty acids, steroids, purines, pyrimidines, derivatives, structural analogs or combinations thereof.

[00157] Candidate agents are obtained from a wide variety of sources including

libraries of synthetic or natural compounds. For example, numerous means are available for random and directed synthesis of a wide variety of organic compounds and biomolecules, including expression of randomized oligonucleotides. Alternatively, libraries of natural compounds in the form of bacterial, fungal, plant and animal extracts are available or readily produced. Additionally, natural or synthetically produced libraries and compounds are readily modified through conventional chemical, physical and biochemical means. Known pharmacological agents may be subjected to directed or random chemical modifications, such as acylation, alkylation, esterification, and/or amidification to produce structural analogs.

[00158] Competitive screening assays may be done by combining KSP and a drug candidate in a first sample. A second sample comprises a compound of the present invention, KSP and a drug candidate. This may be performed in either the presence or absence of microtubules. The binding of the drug candidate is determined for both samples, and a change, or difference in binding between the two samples indicates the presence of a drug candidate capable of binding to KSP and potentially inhibiting its activity. That is, if the binding of the drug candidate is different in the second sample relative to the first sample, the drug candidate is capable of binding to KSP.

[00159] In one embodiment, the binding of the candidate agent to KSP is determined through the use of competitive binding assays. In this embodiment, the competitor is a binding moiety known to bind to KSP, such as an antibody, peptide, binding partner, ligand, etc. Under certain circumstances, there may be competitive binding as between the candidate agent and the binding moiety, with the binding moiety displacing the candidate agent.

[00160] In one embodiment, the candidate agent is labeled. Either the candidate agent, or the competitor, or both, is added first to KSP for a time sufficient to allow binding, if present. Incubations may be performed at any temperature which facilitates optimal activity, typically between 4 and 40°C.

[00161] Incubation periods are selected for optimum activity, but may also be optimized to facilitate rapid high throughput screening. Typically between 0.1 and 1 hour will be sufficient. Excess reagent is generally removed or washed away. The second

component is then added, and the presence or absence of the labeled component is followed, to indicate binding.

[00162] In one embodiment, the competitor is added first, followed by the candidate agent. Displacement of the competitor is an indication the candidate agent is binding to KSP and thus is capable of binding to, and potentially inhibiting, the activity of KSP. In this embodiment, either component can be labeled. Thus, for example, if the competitor is labeled, the presence of label in the wash solution indicates displacement by the agent. Alternatively, if the candidate agent is labeled, the presence of the label on the support indicates displacement.

[00163] In an alternative embodiment, the candidate agent is added first, with incubation and washing, followed by the competitor. The absence of binding by the competitor may indicate the candidate agent is bound to KSP with a higher affinity. Thus, if the candidate agent is labeled, the presence of the label on the support, coupled with a lack of competitor binding, may indicate the candidate agent is capable of binding to KSP.

[00164] Inhibition is tested by screening for candidate agents capable of inhibiting the activity of KSP comprising the steps of combining a candidate agent with KSP, as above, and determining an alteration in the biological activity of KSP. Thus, in this embodiment, the candidate agent should both bind to KSP (although this may not be necessary), and alter its biological or biochemical activity as defined herein. The methods include both in vitro screening methods and in vivo screening of cells for alterations in cell cycle distribution, cell viability, or for the presence, morpohology, activity, distribution, or amount of mitotic spindles, as are generally outlined above.

[00165] Alternatively, differential screening may be used to identify drug candidates that bind to the native KSP, but cannot bind to modified KSP.

[00166] Positive controls and negative controls may be used in the assays. Suitably all control and test samples are performed in at least triplicate to obtain statistically significant results. Incubation of all samples is for a time sufficient for the binding of the agent to the protein. Following incubation, all samples are washed free of non-specifically bound material and the amount of bound, generally labeled agent

determined. For example, where a radiolabel is employed, the samples may be counted in a scintillation counter to determine the amount of bound compound.

[00167] A variety of other reagents may be included in the screening assays. These include reagents like salts, neutral proteins, e.g., albumin, detergents, etc which may be used to facilitate optimal protein-protein binding and/or reduce non-specific or background interactions. Also reagents that otherwise improve the efficiency of the assay, such as protease inhibitors, nuclease inhibitors, anti-microbial agents, etc., may be used. The mixture of components may be added in any order that provides for the requisite binding.

Administration

[00168] Accordingly, the compounds of the invention are administered to cells. By "administered" herein is meant administration of a therapeutically effective dose of a compound of the invention to a cell either in cell culture or in a patient. By "therapeutically effective dose" herein is meant a dose that produces the effects for which it is administered. The exact dose will depend on the purpose of the treatment, and will be ascertainable by one skilled in the art using known techniques. As is known in the art, adjustments for systemic versus localized delivery, age, body weight, general health, sex, diet, time of administration, drug interaction and the severity of the condition may be necessary, and will be ascertainable with routine experimentation by those skilled in the art. By "cells" herein is meant any cell in which mitosis or meiosis can be altered.

[00169] A "patient" for the purposes of the present invention includes both humans and other animals, particularly mammals, and other organisms. Thus the methods are applicable to both human therapy and veterinary applications. In one embodiment the patient is a mammal, and in a more particular embodiment, the patient is human.

[00170] Compounds of the invention having the desired pharmacological activity may be administered, suitably as a pharmaceutically acceptable composition comprising an pharmaceutical excipient, to a patient, as described herein. Depending upon the manner of introduction, the compounds may be formulated in a variety of ways as discussed below. The concentration of therapeutically active compound in the

formulation may vary from about 0.1-100 wt.%.

[00171] The agents may be administered alone or in combination with other treatments, i.e., radiation, or other chemotherapeutic agents such as the taxane class of agents that appear to act on microtubule formation or the camptothecin class of topoisomerase I inhibitors. When used, other chemotherapeutic agents may be administered before, concurrently, or after administration of a compound of the present invention. In one aspect of the invention, a compound of the present invention is co-administered with one or more other chemotherapeutic agents. By "co-administer" it is meant that the present compounds are administered to a patient such that the present compounds as well as the co-administered compound may be found in the patient's bloodstream at the same time, regardless when the compounds are actually administered, including simultaneously.

[00172] The administration of the compounds and compositions of the present invention can be done in a variety of ways, including, but not limited to, orally, subcutaneously, intravenously, intranasally, transdermally, intraperitoneally, intramuscularly, intrapulmonary, vaginally, rectally, or intraocularly. In some instances, for example, in the treatment of wounds and inflammation, the compound or composition may be directly applied as a solution or spray.

[00173] Pharmaceutical dosage forms include a compound of formula I or a pharmaceutically acceptable salt or solvate thereof, and one or more pharmaceutical excipients. As is known in the art, pharmaceutical excipients are secondary ingredients which function to enable or enhance the delivery of a drug or medicine in a variety of dosage forms (e.g.: oral forms such as tablets, capsules, and liquids; topical forms such as dermal, opthalmic, and otic forms; suppositories; injectables; respiratory forms and the like). Pharmaceutical excipients include inert or inactive ingredients, synergists or chemicals that substantively contribute to the medicinal effects of the active ingredient. For example, pharmaceutical excipients may function to improve flow characteristics, product uniformity, stability, taste, or appearance, to ease handling and administration of dose, for convenience of use, or to control bioavailability. While pharmaceutical excipients are commonly described as being inert or inactive, it is appreciated in the art

that there is a relationship between the properties of the pharmaceutical excipients and the dosage forms containing them.

[00174] Pharmaceutical excipients suitable for use as carriers or diluents are well known in the art, and may be used in a variety of formulations. See, e.g., Remington's Pharmaceutical Sciences, 18th Edition, A. R. Gennaro, Editor, Mack Publishing Company (1990); Remington: The Science and Practice of Pharmacy, 20th Edition, A. R. Gennaro, Editor, Lippincott Williams & Wilkins (2000); Handbook of Pharmaceutical Excipients, 3rd Edition, A. H. Kibbe, Editor, American Pharmaceutical Association, and Pharmaceutical Press (2000); and Handbook of Pharmaceutical Additives, compiled by Michael and Irene Ash,Gower (1995), each of which is incorporated herein by reference for all purposes.

[00175] Oral solid dosage forms such as tablets will typically comprise one or more pharmaceutical excipients, which may for example help impart satisfactory processing and compression characteristics, or provide additional desirable physical characteristics to the tablet. Such pharmaceutical excipients may be selected from diluents, binders, glidants, lubricants, disintegrants, colors, flavors, sweetening agents, polymers, waxes or other solubility-retarding materials.

[00176] Compositions for intravenous administration will generally comprise intravenous fluids, i.e., sterile solutions of simple chemicals such as sugars, amino acids or electrolytes, which can be easily carried by the circulatory system and assimilated. Such fluids are prepared with water for injection USP.

[00177] Fluids used commonly for intravenous (IV) use are disclosed in Remington, the Science and Practice of Pharmacy [full citation previously provided], and include:

alcohol (e.g., in dextrose and water ("D/W") [e.g., 5% dextrose] or dextrose and water [e.g., 5% dextrose] in normal saline solution ("NSS"); e.g. 5% alcohol);

synthetic amino acid such as Aminosyn, FreAmine, Travasol, e.g., 3.5 or 7; 8.5; 3.5, 5.5 or 8.5 % respectively;

ammonium chloride e.g., 2.14%;

dextran 40, in NSS e.g., 10% or in D5/W e.g., 10%;

dextran 70, in NSS e.g., 6% or in D5/W e.g., 6%;

dextrose (glucose, D5/W) e.g., 2.5-50%;

dextrose and sodium chloride e.g., 5-20% dextrose and 0.22-0.9% NaCl;

lactated Ringer's (Hartmann's) e.g., NaCl 0.6%, KCl 0.03%, $CaCl_2$ 0.02%;

lactate 0.3%;

mannitol e.g., 5%, optionally in combination with dextrose e.g., 10% or NaCl e.g., 15 or 20%;

multiple electrolyte solutions with varying combinations of electrolytes, dextrose, fructose , invert sugar Ringer's e.g., NaCl 0.86%, KCl 0.03%, $CaCl_2$ 0.033%;

sodium bicarbonate e.g., 5%;

sodium chloride e.g., 0.45, 0.9, 3, or 5%;

sodium lactate e.g., 1/6 M; and

sterile water for injection

The pH of such fluids may vary, and will typically be from 3.5 to 8 such as known in the art.

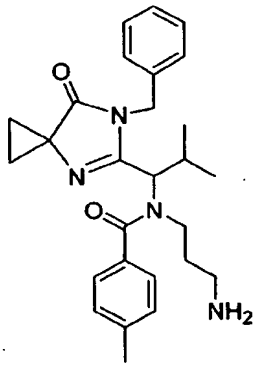
[00178] The following examples serve to more fully describe the manner of using the above-described invention, as well as to set forth the best modes contemplated for carrying out various aspects of the invention. It is understood that these examples in no way serve to limit the true scope of this invention, but rather are presented for illustrative purposes. All publications, including but not limited to patents and patent applications, cited in this specification are herein incorporated by reference as if each individual publication were specifically and individually indicated to be incorporated by reference herein as though fully set forth.

EXAMPLES

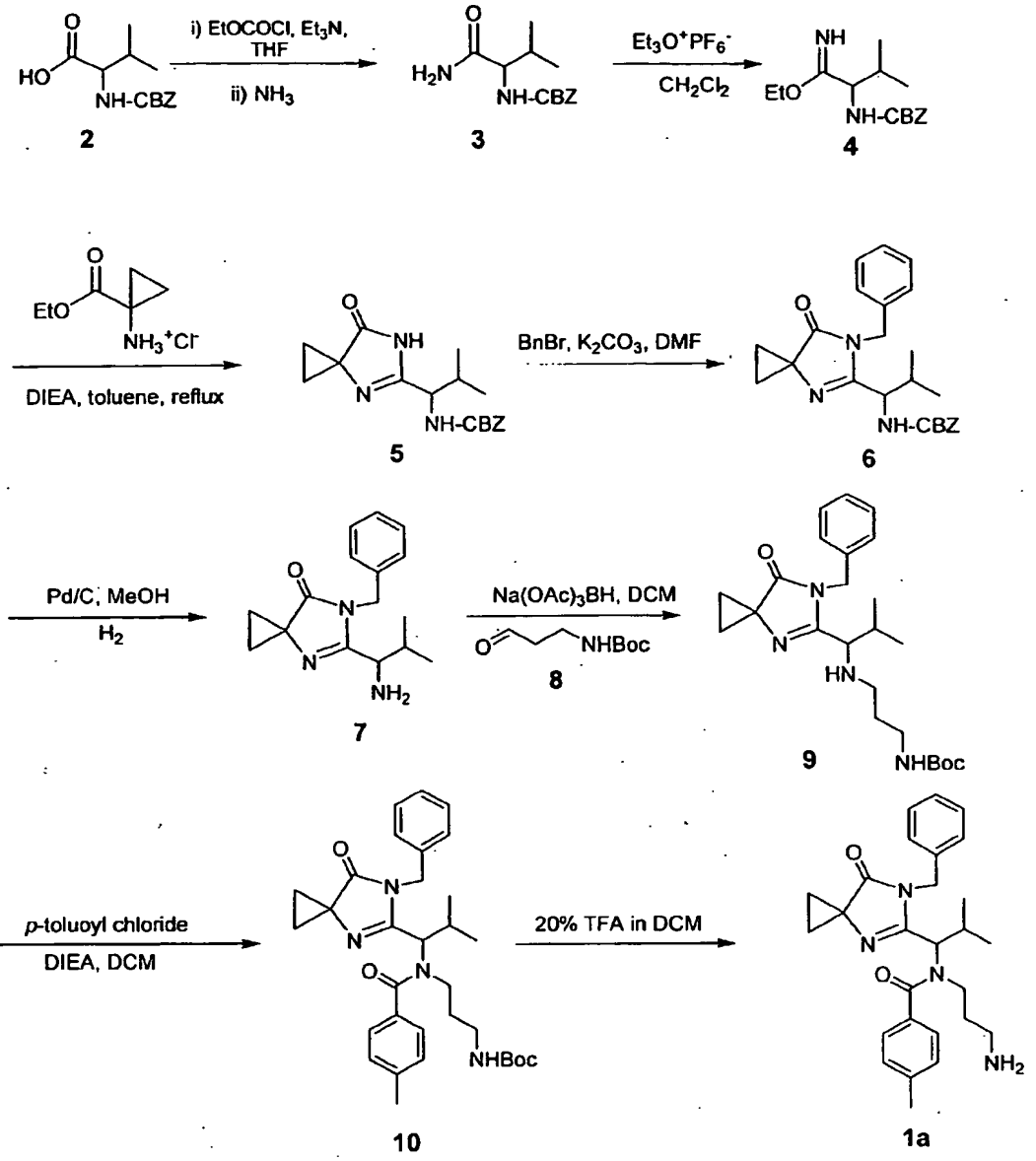
[00179] All anhydrous solvents were purchased from Aldrich Chemical Company in SureSeal® containers.

Example 1

Synthesis of Compound 1a



1a



[00180] To a three-necked 500 mL round bottom flask were added CBZ-Valine (**2**, 50 g, 200 mmol), THF (700 mL), ethyl chloroformate (21 mL, 220 mmol) and triethylamine (33.5 mL, 240 mmol) at 0 °C. The reaction mixture was stirred under nitrogen. After 1 h, the flask was equipped with a dry-ice reflux condenser and purged

continuously with ammonia gas for 30 minutes. The reaction mixture was stirred for an additional 1 h. The reaction mixture was filtered. The precipitate was washed with water and THF, and dried *in vacuo* to give **3** (38 g, 76%) as a white solid. LRMS ($M+H^+$) *m/z* 251.2.

[00181] To a suspension of **3** (20 g, 59 mmol) in dichloromethane (500 mL) was added triethyloxonium hexafluorophosphate (25 g, 100 mmol). The resulting mixture was stirred for 3 days. The reaction mixture was washed with saturated $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated to give **4** (20 g), which was used in the next step without further purification. LRMS ($M+H^+$) *m/z* 279.1.

[00182] To a solution of above crude **4** (2 g, ~7.2 mmol) in toluene (30 mL) were added 1-aminocyclopropane-1-carboxylic acid ethyl ester hydrochloride (594 mg, 3.6 mmol) and *N*, *N*-diisopropylethylamine (625 µL, 3.6 mmol). The resulting mixture was refluxed for 8 h. Acetic acid (200 uL) was added. Reluxing was continued for 2h. The solution was concentrated. The resulting residue was dissolved in dichloromethane (100 mL). The organic layer was washed with water and brine, dried over sodium sulfate, and concentrated. The resulting residue was purified via flash column chromatography using a mixture of ethyl acetate and hexane as eluent to give **5** (210 mg, 18% from **3**). LRMS ($M+H^+$) *m/z* 316.1.

[00183] To a solution of **5** (210 mg, 0.66 mmol) in DMF (5 mL) was added benzyl bromide (102 µl, 0.86 mmol) and K_2CO_3 (182 mg, 1.32 mmol). The resulting mixture was stirred for 3 h and then was diluted with EtOAc (150 mL) and H_2O (50 mL). The organic layer was washed with brine, dried over sodium sulfate, and concentrated to give **6** (270 mg), which was used in the next step without further purification. LRMS ($M+H^+$) *m/z* 406.1.

[00184] A solution of crude **6** (270 mg, ~0.66 mmol) in MeOH (15 mL) was stirred under a stream of H_2 (30 psi) in the presence of 10% Pd/C (20 mg) for 1 h. The catalyst was removed by filtration through a PTFE (0.45 µm) filter and the solvent evaporated to give **7** (180 mg), which was used in the next step without further purification. LRMS ($M+H^+$) *m/z* 272.1.

[00185] To a solution of above crude **7** (180 mg, ~0.66 mmol) in dichloromethane

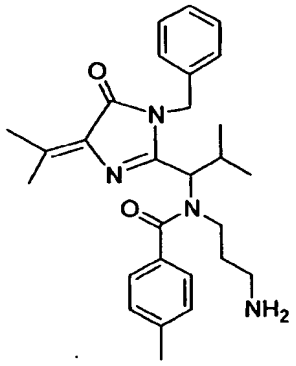
(15 mL) at 0°C was added aldehyde **8** (160 mg, 0.92 mmol) and sodium triacetoxyborohydride (98 mg, 0.46 mmol), successively. The resulting mixture was stirred under nitrogen for 2 h. Additional aldehyde **8** (140 mg, 0.8 mmol) and sodium triacetoxyborohydride (40 mg, 0.19 mmol) were added. Stirring was continued for an additional 1 h. The mixture was diluted with dichloromethane (25 mL) and washed with saturated $NaHCO_3$ (20 mL). The organic layer was separated, and the aqueous phase was extracted with dichloromethane (2 x 25 mL). The combined organic layers were washed with brine, dried over sodium sulfate, and concentrated to give **9** (280 mg), which was used in the next step without purification. LRMS ($M+H^+$) *m/z* 429.2.

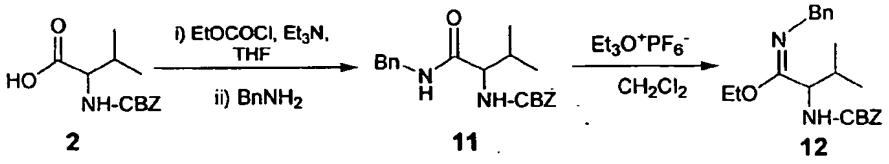
[00186] To a solution of **9** (140 mg, ~0.33 mmol) and *N*, *N*-diisopropylethylamine (150 μL, 0.86 mmol) in dichloromethane (5 mL) was added *p*-toluoyl chloride (132 μL, 1.0 mmol). The resulting solution was stirred for 14 h. The solution was concentrated. The resulting residue was dissolved in dichloromethane (25 mL). It was washed with saturated $NaHCO_3$ (20 mL), and the aqueous phase was extracted with dichloromethane (2 x 20 mL). The combined organic layers were washed with H_2O and brine, dried over sodium sulfate, and concentrated. The resulting residue was purified on RP-HPLC using a mixture of acetonitrile and H_2O to give **10** (50 mg, 28% from **5**). LRMS ($M+H^+$) *m/z* 547.3.

[00187] To a solution of **10** (35 mg, 0.064 mmol) in dichloromethane (20 mL) was added trifluoroacetic acid (2 mL). The resulting solution was stirred at room temperature for 1 h and then concentrated under reduced pressure. The residue was dried under high vacuum and dissolved in ethyl acetate (25 mL). It was washed with saturated $NaHCO_3$ (20 mL), and the aqueous phase was extracted with ethyl acetate (2 x 25 mL). The combined organic layers were dried over sodium sulfate, and concentrated to give **1a** as a white solid (25 mg, 88%), which was fully characterized with ^{1}H-NMR and LC/MS analysis (LRMS ($M+H^+$) *m/z* 447.2).

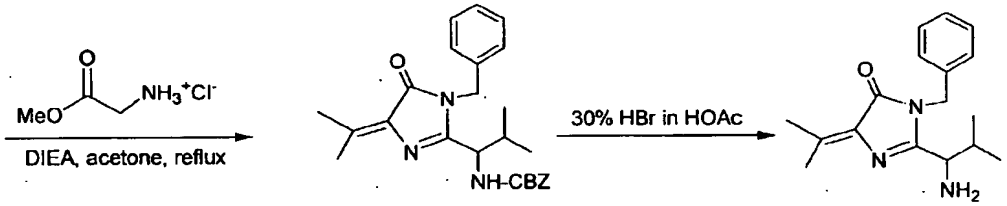
Example 2

Synthesis of Compound 1b

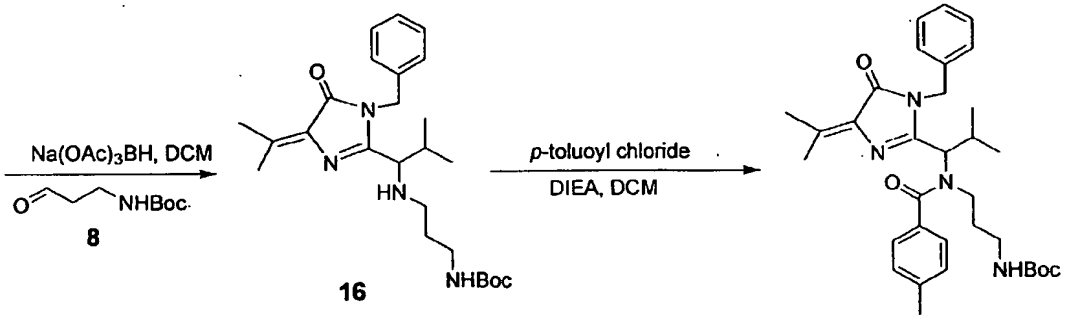




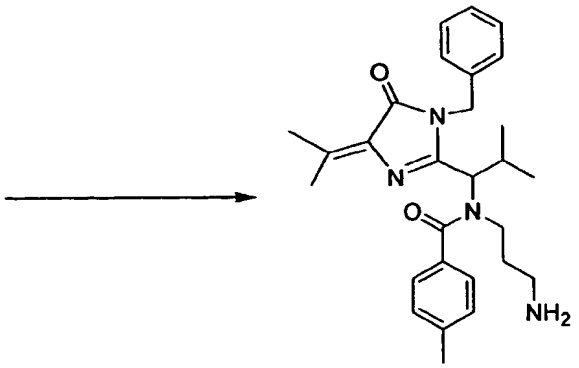
2 11 12



14 15



8 16 17



1b

[00188] To a solution of CBZ-Valine (**2**, 50 g, 200 mmol) in THF (700 mL) were added ethyl chloroformate (23 mL, 240 mmol) and triethylamine (33.5 mL, 240 mmol) at 0 °C. The reaction micxture was stirred under nitrogen. After 1 h, benzylamine (26.2 mL, 240 mmol) was added over 5 minutes. Upon completion of addition, the reaction solution was allowed to warm to room temperature. After 1 h, the reaction mixture was filtered. The precipitate was washed with water and THF, and dried *in vacuo* to give **11** (60 g, 88%) as a white solid. LRMS ($M+H^+$) *m/z* 341.1.

[00189] To a suspension of **11** (20 g, 59 mmol) in dichloromethane (500 mL) was added triethyloxonium hexafluorophosphate (25 g, 100 mmol). The resulting mixture was stirred for 14 h. The reaction mixture was washed with saturated $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated to give **12** (19 g), which was used in the next step without further purification. LRMS ($M+H^+$) *m/z* 369.1.

[00190] To a solution of above crude **12** (2.8 g, ~7.6 mmol) in acetone (30 mL) were added glycine methyl ester hydrochloride (1.9 g, 15.2 mmol) and *N*, *N*-diisopropylethylamine (1.97 mL, 11.3 mmol). The resulting mixture was refluxed for 20 h. The solution was concentrated. The resulting residue was dissolved in EtOAc (300 mL). The organic layer was washed with water and brine, dried over sodium sulfate, and concentrated. The resulting residue was purified on RP-HPLC using a mixture of acetonitrile and H_2O to give **14** (250 mg, 8% from **11**). LRMS ($M+H^+$) *m/z* 420.1.

[00191] A solution of crude **14** (100 mg, 0.24 mmol) in HOAc containing 30% HBr (2.3 mL) was stirred for 1 h. The reaction mixture was concentrated and dried *in*

vacuo to give **15** (70 mg), which was used in the next step without further purification. LRMS ($M+H^+$) *m/z* 286.3.

[00192] To a solution of above crude **15** (70 mg, ~0.24 mmol) in dichloromethane (15 mL) at 0°C was added aldehyde **8** (50 mg, 0.29 mmol) and sodium triacetoxyborohydride (40 mg, 0.19 mmol), successively. The resulting mixture was stirred under nitrogen for 2 h. Additional aldehyde **8** (50 mg, 0.29 mmol) and sodium triacetoxyborohydride (20 mg, 0.1 mmol) were added. Stirring was continued for an additional 2 h. The mixture was diluted with dichloromethane (25 mL) and saturated $NaHCO_3$ (20 mL). The organic layer was separated, and the aqueous phase was extracted with dichloromethane (2 x 25 mL). The combined organic layers were washed with brine, dried over sodium sulfate, and concentrated to give **16** (150 mg), which was used in the next step without purification. LRMS ($M+H^+$) *m/z* 443.5.

[00193] To a solution of **16** (150 mg, ~0.24 mmol) and *N*, *N*-diisopropylethylamine (124 μL, 0.71 mmol) in dichloromethane (15 mL) was added *p*-toluoyl chloride (100 μL, 0.71 mmol). The resulting solution was stirred for 14 h. The solution was concentrated. The resulting residue was purified on RP-HPLC using a mixture of acetonitrile and H_2O to give **17** (20 mg, 15% from **14**). LRMS ($M+H^+$) *m/z* 561.6.

[00194] To a solution of **17** (20 mg, 0.036 mmol) in dichloromethane (6 mL) was added trifluoroacetic acid (1.5 mL). The resulting solution was stirred at room temperature for 2 h and then concentrated under reduced pressure. The resulting residue was purified on RP-HPLC using a mixture of acetonitrile and H_2O. The resulting product was dissolved in dichloromethane (20 mL). It was washed with saturated $NaHCO_3$ (20 mL), and the aqueous phase was extracted with dichloromethane (2 x 20 mL). The combined organic layers were dried over sodium sulfate, and concentrated to give **1b** as a white solid (6 mg, 37%), which was fully characterized with ^{1}H-NMR and LC/MS analysis (LRMS ($M+H^+$) *m/z* 461.5).

Example 3

Monopolar Spindle Formation following Application of a KSP Inhibitor

[00195] Human tumor cells Skov-3 (ovarian) were plated in 96-well plates at densities of 4,000 cells per well, allowed to adhere for 24 hours, and treated with various concentrations of the KSP inhibitors for 24 hours. Cells were fixed in 4% formaldehyde and stained with antitubulin antibodies (subsequently recognized using fluorescently-labeled secondary antibody) and Hoechst dye (which stains DNA).

[00196] Visual inspection revealed that the compounds caused cell cycle arrest in the prometaphase stage of mitosis. DNA was condensed and spindle formation had initiated, but arrested cells uniformly displayed monopolar spindles, indicating that there was an inhibition of spindle pole body separation. Microinjection of anti-KSP antibodies also causes mitotic arrest with arrested cells displaying monopolar spindles.

Example 4

Inhibition of Cellular Proliferation in Tumor Cell Lines

[00197] Cells were plated in 96-well plates at densities from 1000-2500 cells/well of a 96-well plate and allowed to adhere/grow for 24 hours. They were then treated with various concentrations of drug for 48 hours. The time at which compounds are added is considered T_0. A tetrazolium-based assay using the reagent 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) (I.S> Patent No. 5,185,450) (see Promega product catalog #G3580, CellTiter 96® AQ_{ueous} One Solution Cell Proliferation Assay) was used to determine the number of viable cells at T_0 and the number of cells remaining after 48 hours compound exposure. The number of cells remaining after 48 hours was compared to the number of viable cells at the time of drug addition, allowing for calculation of growth inhibition.

[00198] The growth over 48 hours of cells in control wells that had been treated with vehicle only (0.25% DMSO) is considered 100% growth and the growth of cells in wells with compounds is compared to this. KSP inhibitors inhibited cell proliferation in human ovarian tumor cell lines (SKOV-3).

[00199] A Gi_{50} was calculated by plotting the concentration of compound in μM vs the percentage of cell growth of cell growth in treated wells. The Gi_{50} calculated for the compounds is the estimated concentration at which growth is inhibited by 50% compared

to control, i.e., the concentration at which:

$$100 \times [(Treated_{48} - T_0) / (Control_{48} - T_0)] = 50.$$

[00200] All concentrations of compounds are tested in duplicate and controls are averaged over 12 wells. A very similar 96-well plate layout and Gi_{50} calculation scheme is used by the National Cancer Institute (see Monks, et al., J. Natl. Cancer Inst. 83:757-766 (1991)). However, the method by which the National Cancer Institute quantitates cell number does not use MTS, but instead employs alternative methods.

Example 5

Calculation of IC_{50}:

[00201] Measurement of a composition's IC_{50} for KSP activity uses an ATPase assay. The following solutions are used: Solution 1 consists of 3 mM phosphoenolpyruvate potassium salt (Sigma P-7127), 2 mM ATP (Sigma A-3377), 1 mM DTT (Sigma D-9779), 5 μM paclitaxel (Sigma T-7402), 10 ppm antifoam 289 (Sigma A-8436), 25 mM Pipes/KOH pH 6.8 (Sigma P6757), 2 mM MgC12 (VWR JT400301), and 1 mM EGTA (Sigma E3889). Solution 2 consists of 1 mM NADH (Sigma N8129), 0.2 mg/ml BSA (Sigma A7906), pyruvate kinase 7U/ml, L-lactate dehydrogenase 10 U/ml (Sigma P0294), 100 nM KSP motor domain, 50 μg/ml microtubules, 1 mM DTT (Sigma D9779), 5 μM paclitaxel (Sigma T-7402), 10 ppm antifoam 289 (Sigma A-8436), 25 mM Pipes/KOH pH 6.8 (Sigma P6757), 2 mM MgC12 (VWR JT4003-01), and 1 mM EGTA (Sigma E3889). Serial dilutions (8-12 two-fold dilutions) of the composition are made in a 96-well microtiter plate (Corning Costar 3695) using Solution 1. Following serial dilution each well has 50 μl of Solution 1. The reaction is started by adding 50 μl of solution 2 to each well. This may be done with a multichannel pipettor either manually or with automated liquid handling devices. The microtiter plate is then transferred to a microplate absorbance reader and multiple absorbance readings at 340 nm are taken for each well in a kinetic mode. The observed rate of change, which is proportional to the ATPase rate, is then plotted as a function of the compound concentration. For a standard IC_{50} determination the data acquired is fit by the following four parameter equation using a nonlinear fitting program (e.g., Grafit 4):

$$y = \frac{\text{Range}}{1 + \left(\frac{x}{IC_{50}}\right)^{s}} + \text{Background}$$

where y is the observed rate and x the compound concentration.

[00202] Other compounds of this class were found to inhibit cell proliferation, although GI_{50} values varied. GI_{50} values for the compounds tested ranged from 200 nM to greater than the highest concentration tested. By this we mean that although most of the compounds that inhibited KSP activity biochemically did inhibit cell proliferation, for some, at the highest concentration tested (generally about 20 μM), cell growth was inhibited less than 50%. Many of the compounds have GI_{50} values less than 10 μM, and several have GI_{50} values less than 1 μM. Anti-proliferative compounds that have been successfully applied in the clinic to treatment of cancer (cancer chemotherapeutics) have GI_{50}'s that vary greatly. For example, in A549 cells, paclitaxel GI_{50} is 4 nM, doxorubicin is 63 nM, 5-fluorouracil is 1 μM, and hydroxyurea is 500 μM (data provided by National Cancer Institute, Developmental Therapeutic Program, http://dtp.nci.nih.gov/). Therefore, compounds that inhibit cellular proliferation at virtually any concentration may be useful.

COMPOUNDS, COMPOSITIONS, AND METHODS

ABSTRACT OF THE INVENTION

Compounds useful for treating cellular proliferative diseases and disorders by inhibiting the activity of KSP are disclosed.